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Cardiorenal interaction : experimental and clinical studies

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CARDIORENAL INTERACTION: EXPERIMENTAL AND CLINICAL STUDIES

Mariusz K. Szymański

Cardiorenal interaction:
experimental and clinical studies

Mariusz Krzysztof Szymański

Cardiorenal interaction: experimental and clinical studies
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U M C G

Stellingen

Behorende bij het proefschrift

Cardiorenal interaction: experimental and clinical studies

Door Mariusz K. Szymański

1. There is an urgent need for better animal models to study the cardiorenal interaction. (dit proefschrift).

2. There are other mechanisms than only cardiac remodeling that lead to increased sensitivity to cardiac ischemia in the presence of reduced kidney function (dit proefschrift).

3. Endothelial dysfunction plays an intermediate role in the interaction between heart and kidney in a new animal model for cardiorenal interaction (dit proefschrift).

4. ^{13}N-NH_3 micro-PET can be used as a non-invasive method for assessment of the cardiorenal axis in experimental models (dit proefschrift).

5. Excessive increase in renin concentration during treatment with ACE inhibitors has a prognostic value in heart failure patients with decreased kidney function (dit proefschrift).

6. Voor welke klus dan ook, staat ergens in dit land een Pool klaar. (zo ook voor dit proefschrift)

7. Net als bij een hardloopwedstrijd is bij promotieonderzoek de weg naar de eindstreep langer dan verwacht en 'vals plat'.

8. My home is where my bike is.

9. The distance from The Hague to Groningen seems to be significantly longer than from Groningen to The Hague.

10. Homage to thee, O my heart! Homage to you, O my kidneys! (Book of the Dead, 1600-1240 BC)

11. Science is facts; just as houses are made of stones, so is science made of facts; but a pile of stones is not a house and a collection of facts is not necessarily science (Henri Poincare)

12. The roots of education are bitter, but the fruit is sweet (Aristotle)

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experimental and clinical studies

Proefschrift

ter verkrijging van het doctoraat in de
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door

Mariusz Krzysztof Szymański

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To my Parents /

Rodzicom

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Chapter 1

General introduction

Introduction

Heart failure is a complex clinical syndrome characterized by reduced cardiac function and increased venous congestion. According to recent data, the prevalence is estimated at approximately 1-2% of the general population [1]. Despite the introduction of many treatment modalities over the past decades, heart failure related morbidity and mortality rates remain high [1]. This may be explained by several factors, such as better survival following myocardial infarction, advanced ageing of heart failure population, as well as the presence of the common co-morbidities, which limit the efficacy of heart failure treatment and worsen overall prognosis. Heart failure is often accompanied by dysfunction of other organs, such as the endothelium, the kidney, the liver and the bone marrow. Renal dysfunction in heart failure has received increasing attention in last years [2,3]. The co-existence of these entities has been shown to further increase the risk for mortality and hospitalizations [4]. Interestingly, the extent of kidney dysfunction has been shown to predict the outcome of heart failure patients more accurately than cardiac contractile function (measured by left ventricular ejection fraction) itself [5]. Moreover, renal dysfunction has been shown an independent risk factor for the development of cardiovascular diseases and has been associated with poor outcome in a broad spectrum of cardiovascular patients [6-9]. Conversely, cardiac dysfunction is more likely to develop in patients with chronic renal dysfunction and leads to further decrease in renal function [10]. This interaction between heart and kidney, where dysfunction of either one of them leads to disorder of the other, is usually referred to as the cardiorenal syndrome [11].

Cardiorenal syndrome

It has been suggested that cardiac and renal dysfunction influence each other through shared pathological pathways [11,12]. Hemodynamic changes are considered the main driving force in the pathophysiology of the cardiorenal syndrome. A decrease in cardiac output, a hallmark of heart failure, below the autoregulatory range, leads to a decreased renal blood flow (RBF), which is the main determinant of decreased glomerular filtration rate (GFR) in heart failure population [13]. Moreover, recent studies have shown that not only forward failure but also venous congestion plays an important role in the pathophysiology of renal dysfunction in heart failure. Increased central venous pressure has been shown to be associated with impaired renal function and to be independently related to all cause mortality [14].

Upon the appearance of the above described hemodynamic changes, multiple regulatory mechanisms are activated, including the renin-angiotensin-aldosterone system (RAAS), the sympathetic nervous system (SNS) and the nitric oxide/reactive oxygen species balance [11,12]. The activation of RAAS has been suggested to be of special interest. Initially aimed to preserve cardiac and renal homeostasis, long-term activation of the RAAS eventually leads to changes in the myocardium and the progression of heart failure as well as salt and water retention with increased congestion and, as a consequence, further reduction in renal blood

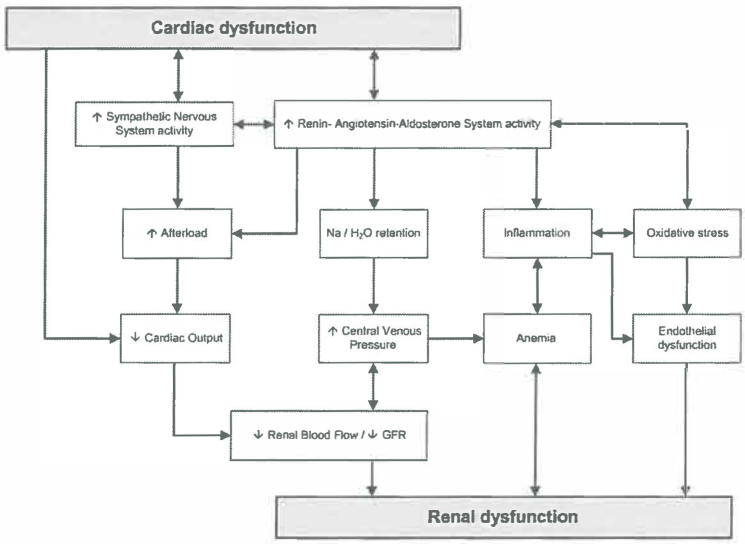

Figure 1. *Pathophysiological connections in the cardiorenal syndrome (Adapted from Bock et al.[17])*

flow. Activated RAAS interacts also with other cardiorenal connectors, such as SNS and the oxidative stress cascade [15,16]. Recent studies have underscored the important role of other newly identified factors such as anemia and endothelial dysfunction (figure 1). Because of close interaction between different pathophysiological mechanisms involved in the cardiorenal axis, activation of one of such factors often effect the others. Like with the RAAS, activation of these regulatory systems, at first compensatory, eventually leads on the long term to a vicious circle resulting in further structural and functional damage in heart and/or kidney.

However, despite new insights in the pathophysiology of the cardiorenal syndrome, the complex heart-kidney interactions are still only partially understood. Although we have gained insight in factors predicting outcome in heart failure patients with renal dysfunction, we are currently not able to define specific cardiorenal treatment strategies in heart failure patients. This could also be addressed by findings in the recent clinical trials in heart failure patients, in which no improvement in renal function has been shown upon different treatments [18,19,20]. Therefore, further research is needed to better understand the cardiorenal syndrome and improve treatment strategies in this patient population..

Experimental studies on cardiorenal interaction

Because of the observational character, post-hoc conclusions obtained from clinical studies have not led to new insights and treatments for the pathophysiology of cardiorenal interaction. This may be achieved by well-designed animal studies. The main requirements for such studies are: use of the proper animal model and use of techniques that allow studying important aspects of this interaction.

There are few rodent models described in literature, which have been used in studies on cardiorenal interaction [21-24]. However, none of the models entirely reproduce the pathophysiology and characteristics of the cardiorenal syndrome observed in humans. Therefore, future research should focus on developing a better model, which would mimic clinical characteristics of this syndrome by cardiac, renal, hemodynamic, and neurohumoral alterations, and allow the evaluation of various treatment strategies.

Not only a relevant model is needed for the adequate study on cardiorenal interaction, but also techniques that will allow assessment of the cardiorenal connectors in this model. Whereas the activation of various systems, such as RAAS or SNS, can be quantified with the use of available plasma and tissue assessments, the proper assessment of hemodynamic changes remains a challenge. Introduction of various imaging techniques into preclinical research has already significantly improved our understanding of the global hemodynamic changes observed in cardiorenal axis. However, the quantification of regional organ perfusion still remains a challenge. The techniques used now are highly invasive and thus do not allow repetitive measurements. Therefore, there is a need for a noninvasive, preclinical method for the evaluation of cardiac and renal perfusion and research should focus on developing such a method.

RAAS blockade in the cardiorenal syndrome

The described above interactions between various cardiorenal connectors lead not only to a vicious circle and deterioration of function of both organs, but may also substantially modulate the effectiveness of common treatment strategies used in heart failure. Drugs targeted to block one of the systems, may also, via feedback loops or compensatory mechanisms, yield an effect on other factors and systems involved in the cardiorenal interaction, which can limit the effects of the treatment. As an example, one of the most commonly used treatment strategies in cardiorenal patients is blocking of the RAAS. This has been proven to reduce heart failure-centered morbidity and mortality [25-26]. On the other hand, although initially helping to preserve GFR to some extent [27], long-term RAAS blockade substantially disables regulatory mechanisms of the kidney [13] and can lead to further decrease in renal blood flow and GFR. Moreover RAAS-blocking agents increase the plasma levels of prorenin, renin and plasma renin activity (PRA). Although initially believed to be unharmful, more data are available suggesting they should be considered prognostic factors in the heart failure population [28,29]. Taken into consideration the new findings, the efficacy of RAAS blockade and its effect on neurohormonal status and prognosis should be studied in patients without and with concomitant renal disease.

Aims of the thesis

The aim of this thesis was to study various aspects of the pathophysiology of the cardiorenal syndrome in both experimental and clinical setting.

Part I focuses on the animal models available for the experimental studies on the interaction between heart and kidney. **Chapter 2** provides a review of the different animal models that have been used so far to study this interaction. In **chapter 3** and **chapter 4** we describe an animal model of combined heart and kidney dysfunction that might serve as a tool for experimental studies on cardiorenal interaction.

In **Part II** we investigated the possibilities of imaging of cardiorenal axis in animal models with the use of ^{13}N-NH_3 micro-PET. **In chapter 5** we explored the possibilities to measure left ventricle parameters with this method, whereas **chapter 6** focuses on the measurements of cardiac and renal regional perfusion.

Finally, in **Part III** the role of activation and blockade of the RAAS in the cardiorenal interaction has been investigated in the clinical setting. In **chapter 7** we studied the prognostic role of renin and prorenin in heart failure patients treated with RAAS blocking agents.

In **chapter 8** the findings reported in this thesis are summarized and put into the future perspective.

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Chapter 2

Animal models of cardiorenal interaction. A review

Mariusz K. Szymański, Rudolf A. de Boer,
Gerjan J. Navis, Wiek H. van Gilst,
Hans L. Hillege

Heart Fail Rev; 2011 Sep 10.

Abstract

The incidence of heart failure and renal failure is increasing and is associated with poor prognosis. Moreover, these conditions do often coexist and this coexistence results in worsened outcome. Various mechanisms have been proposed as an explanation of this interrelation, including changes in hemodynamics, endothelial dysfunction, inflammation, activation of renin-angiotensin-aldosterone system, and/or sympathetic nervous system. However, the exact mechanisms initializing and maintaining this interaction are still unknown. In many experimental studies on cardiac or renal dysfunction, the function of the other organ was either not addressed or the authors failed to show any decline in its function despite histological changes. There are few studies in which the dysfunction of both heart and kidney function has been described. In this review, we discuss animal models of combined cardiorenal dysfunction. We show that translation of the results from animal studies is limited, and there is a need for new and better models of the cardiorenal interaction to improve our understanding of this syndrome. Finally, we propose several requirements that a new animal model should meet to serve as a tool for studies on the cardiorenal syndrome.

Introduction

Recent observations from clinical trials have enhanced the interest in the interaction between heart and kidney. Renal dysfunction has been shown an independent risk factor for the development of cardiovascular (CV) diseases [1, 2] and is associated with worsened outcome in patients with hypertension [3], post myocardial infarction (MI) [4, 5], and a broad spectrum of patients with left ventricular dysfunction [6, 7]. Moreover, in chronic renal failure, CV morbidities are the main cause of mortality. Conversely, cardiac dysfunction, for instance post-MI, leads to a gradual decrease in renal function as reflected by an increase in creatinine levels [8]. This interaction between heart and kidney, where dysfunction of either one of them leads to disorder of the other, is usually referred to as the cardiorenal syndrome. It has been proposed that mechanisms of this organ crosstalk include various changes in hemodynamics, dysregulation of salt and fluid balance, endothelial dysfunction, inflammation, and activation of regulatory systems such as the renin-angiotensin-aldosterone system (RAAS), and sympathetic nervous system (SNS) [9, 10]. The described alterations may disturb other factors and lead to a vicious circle, resulting in further structural and functional damage in heart and/or kidney. Commonly used drugs such as RAAS blockers or beta-blockers may affect not only the targeted system, but may also cause, via feedback loops or compensatory mechanisms, an increase in other factors involved in the cardiorenal interaction. However, the exact pathophysiological mechanisms behind the cardiorenal syndrome still remain unclear. The design of most clinical studies on the cardiorenal interaction does not allow drawing conclusions and explanations for the heart-kidney interaction. Alternatively, this may be achieved by well-designed animal studies. Many animal studies on cardiac or renal dysfunction have been performed and described in literature. However, the authors either did not address the function of the other organ of interest or the models they used did not mimic the characteristics of the clinical cardiorenal syndrome. There are also few animal models described, which combine cardiac and renal dysfunction. The objective of this review is to discuss these combined animal models used in studies on cardiorenal interaction. To this purpose, we will briefly describe the main pathophysiological characteristics of chronic cardiorenal failure and then discuss the available animal models. Because an adequate animal model would be instrumental for better understanding of this important clinical condition, we also discuss the need for a new model and characteristics of a new model, which would help to study the pathophysiology of the cardiorenal syndrome.

Pathophysiology of the cardiorenal interaction

Cardiorenal interaction is usually defined as a disorder of heart and kidney where dysfunction of one of the organs induces disorder of the other. Several pathophysiological mechanisms have been proposed to underlie the interaction between heart and kidney in the cardiorenal

syndrome [9-12]. Guyton [13] described a model of complex hemodynamic connections between heart and kidney. Bongartz et al. [9] proposed a model based on Guyton's model and extended it by 4 cardiorenal connectors responsible for the progression of the cardiorenal syndrome: the RAAS, the SNS, inflammation, and nitric oxide/reactive oxygen species (ROS) balance. Hemodynamic changes are considered the main driving force in the pathophysiology of the cardiorenal syndrome. If the failing heart cannot maintain cardiac output, this results in reduction in perfusion of peripheral organs [11], including decrease in renal blood flow (RBF). Decreased renal perfusion has been shown the main determinant of reduced glomerular filtration rate (GFR) in heart failure patients [14]. Recent publications have shown that GFR reduction in heart failure patients is also inversely related to central venous pressure (CVP). In the ESCAPE trial, the only hemodynamic parameter associated with renal insufficiency was right atrial pressure [15], suggesting an important role of (renal) congestion. Increased CVP in the situation of unchanged systemic arterial pressure leads to decrease in pressure gradient across the glomeruli and subsequent decrease in RBF, but also to increased hydrostatic pressure in the kidney and subsequently to hypoxia and/or activation of intrarenal RAAS. It has been suggested that the contribution of CVP to kidney dysfunction is independent of cardiac output; however, it occurs only when forward failure is present [16]. The relationship between CVP and GFR seems to be bidirectional, as impairment in renal function may lead to salt and water retention and thus to increased venous pressures [17].

Hemodynamic changes in heart failure activate multiple regulatory mechanisms, among which the RAAS is the most important system. Initially aimed to preserve cardiac homeostasis, long-term activation of the RAAS eventually leads to the progression of HF [17], myocardial remodeling [18], and fibrosis and necrosis in the myocardium [19]. Blocking RAAS reverses histological changes in the myocardium, improves endothelial function, and reduces adrenergic tone, resulting in improved prognosis. Less is however known about the effects of long-term RAAS inhibition on renal function in HF. It may help to preserve GFR to some extent [20]; however, long-term RAAS blockade substantially disables regulatory mechanisms of the kidney [14]. Progression of heart failure leads also to compensatory activation of baroreceptors and increase in sympathetic activity. This on one hand preserves the cardiac output, but has also adverse effects such as sympathetic overdrive, cardiomyocyte apoptosis and necrosis, and arrhythmias on the long term. Sympathetic activation has been also suggested to have direct vascular effects on renal vasculature, as renal sympathetic denervation may improve GFR [21]. Moreover, the activated SNS interacts with other cardiorenal connectors, such as the RAAS [22, 23] and the oxidative stress cascade [9].

There is growing evidence that one of the connectors of the cardiorenal syndrome is oxidative stress. In heart failure patients, increased oxidative stress has been demonstrated [24] and impaired NO-mediated endothelial vasodilatation has been related to reduction in renal perfusion [25]. Lower availability of NO and increased production of ROS lead to endothelial dysfunction that has been related to reduction in renal perfusion [25], inflammation, and organ

damage. Inflammation is considered a risk factor for incidence of MI and death in uremic patients [26] and a marker of severity and progression of heart failure [27–30]. Moreover, interaction between oxidative stress, inflammation, and activation of RAAS has been reported [31, 32].

Another observed phenomenon of the relationship between heart and renal failure is anemia [33]. Presence of anemia has been shown to be associated with poor prognosis in heart failure [34]. The pathogenesis of anemia in heart failure seems to be multifactorial and to some extent is a result of decreased renal function [35, 36]. The combination of anemia and renal dysfunction in heart failure patients is associated with much worse outcome suggesting interaction between those two entities [37, 38]. Correction of anemia improves cardiac performance in patients with chronic kidney disease [39, 40] and both cardiac and renal function in heart failure [41, 42].

The vicious circle of pathophysiological changes in cardiorenal interaction leads to dysfunction of one or both organs, accompanied by structural changes in both heart and kidney. Pathological cardiac remodeling includes changes in tissue architecture and myocyte/capillary ratio, increased fibrosis and apoptosis, and occurs not only in response to cardiac, but also to renal stimuli. Presence of albuminuria as a sign of glomerular injury has been shown to be a strong predictor of CV outcome in the general population [43] and in patients with hypertension [44]. However, the pathophysiology of albuminuria and its relation with prognosis is unclear in heart failure. There is also evidence that tubular damage is present in HF population [45] and is associated with prognosis [46]. The pathophysiological mechanisms underlying tubular damage in heart failure are still unknown, but possible explanations include regional hypoxia due to decreased RBF and diuretic therapy. Pathophysiological connections behind the cardiorenal interactions are presented in figure 1, and the most important clinical characteristics of this syndrome are summarized in table 1.

Table 1. *Pathophysiological characteristics of cardiorenal syndrome*

Characteristics	Clinical manifestation
Forward failure	Decreased cardiac output Reduced RBF
Backward failure	Increased Venous Congestion
Neurohormonal activation	Activation of RAAS Activation of SNS
Oxidative stress	Lower availability of nitric oxide Increased production of ROS
Inflammation	Circulating cytokines
Endothelial dysfunction	
Anemia	Low hemoglobine / hematocit levels

RBF, renal bloof flow; RAAS, renin-angiotensin-aldosterone system; SNS, sympathetic nervous system; ROS, reactive oxygen species

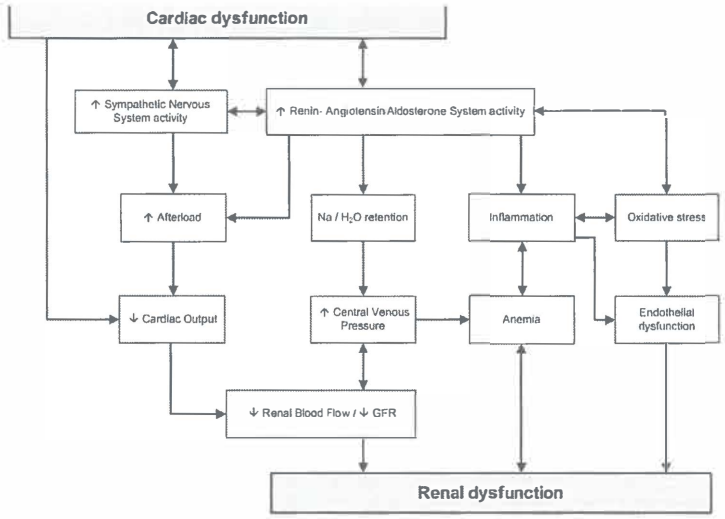

Figure 1. Pathophysiological connections in the cardiorenal syndrome (adapted from Bock et al. [12])

Animal models for cardiorenal interaction

Only a few animal models have been proposed so far as a model for further studies on the cardiorenal syndrome in which both cardiac and renal dysfunction have been induced (Table 2). They are all based on the use of rodents. Advantages of small animals include lower costs, availability, and housing conditions. There are multiple ways of inducing cardiac dysfunction in rodent models. They comprise of volume or pressure overload, myocardial ischemia/infarction, administration of toxic agents, and rapid ventricular pacing.

The most commonly used technique of inducing cardiac dysfunction in studies on the cardiorenal interaction is MI resulting from ligation of the left coronary artery. Main advantages of this model are simplicity, reproducibility, and its relation to human pathophysiology. In this model, the cardiac output is significantly reduced 3–5 weeks after surgery [47] and the impairment of left ventricular function is directly related to the loss of myocardium [48]. Hemodynamic and neurohormonal changes in this model resemble those of heart failure observed in humans [49] and are also dependent on the extent of cardiac damage [50–52]. The use of the MI model has been essential in establishing the beneficial effects of RAAS blockade [53, 54], which were later confirmed in clinical trials, underscoring the agreement between this model and human heart failure with respect to the pathophysiology and treatment possibilities.

The most commonly used techniques of inducing renal failure are based on the reduction of viable kidney tissue in different ways. The main difference between them is the extent of the removal and thus the degree of renal dysfunction they cause. Unilateral nephrectomy (UNX) leads to mild renal function impairment without a substantial increase in proteinuria or histological changes [55], whereas subnephrectomy (SNX) leads to more severe renal

Table 2. Rodent models of combined cardiorenal failure

Model	Reference	Cardiac changes	Renal changes
UNX+MI	Van Dokkum [55]	Increased SBP Decreased dP/dt max and dP/dt min LVH	Increased plasma creatinine Increased proteinuria FGS
SNX+MI	Windt [57]	Increased SBP Decreased dP/dt max and dP/dt min LVH Decreased capillary density	Decreased creatinine clearance Decreased renal blood flow Increased proteinuria FGS Fibrosis
	Dikow [62]	Increased SBP Higher susceptibility to myocardial ischemia	Increased serum urea Increased serum creatinine
	Dikow [56]	Increased SBP Decreased EF Increased LVEDD LVH Fibrosis Decreased capillary density	Increased serum urea Increased serum creatinine
SNX+L-NNA	Bongartz [66]	Increased blood pressure Decreased EF Increased LVEDV LVH Fibrosis	Increased plasma creatinine Increased plasma urea Decreased creatinine clearance Increased proteinuria FGS Tubulo-interstitial injury
Adriamycin	Noiri [70]	LVH	Increased plasma creatinine Increased plasma urea Decreased creatinine clearance Fibrosis Tubular damage

UNX, uninephrectomy; SNX, subnephrectomy; MI, myocardial infarction; L-NNA, Nω-nitro-L-arginine, SBP, systolic blood pressure; LVH, left ventricle hypertrophy; EF, ejection fraction; LVEDD, left ventricle end-diastolic diameter; LVEDV, left ventricle end-diastolic volume; FGS, focal glomerulosclerosis;

dysfunction and eventually to uremia and chronic kidney disease complications similar as in humans [56, 57]. Changes in cardiac tissue architecture have also been reported in subnephrectomized animals [58–60].

Van Dokkum et al. [55] have described a model of UNX followed by MI 1 week later. They have shown an accelerated renal dysfunction in uninephrectomized rats when combined with cardiac dysfunction. This has been evidenced by increased levels of proteinuria. Also more renal damage, as measured by focal glomerulosclerosis, has been reported in animals with combined cardiac and renal dysfunction. Creatinine levels were increased, as expected, after nephrectomy; however, addition of MI did not lead to further increase. Interestingly, the decrease in renal function was more pronounced in animals with large infarcts. This might be due to the severity of cardiac dysfunction and its effect on renal hemodynamics. It has been shown that in rats after MI, the severity of ventricular dysfunction and subsequent hemodynamic changes depend on infarct size [48, 61] as well as time between intervention and measurements [47, 52]. Decrease in cardiac function due to small and moderate infarcts, results in either small or no decrease in RBF [50–52]. When RBF is minimally decreased, the animal upkeeps its GFR by a compensatory increase in renal vascular resistance, like in humans [51]. More severe cardiac dysfunction as a result of large infarction leads to a larger fall in RBF and a subsequent decrease in GFR despite further increase in venous resistance [51, 52]. However, the data on RBF in this model are missing, and the only indication of more severe cardiac dysfunction in the group with larger MI was more advanced hypertrophy of the heart. No differences in brain natriuretic peptide (BNP) levels, LV pressures nor contractility or relaxation between groups have been reported. The blood pressure was higher in the animals with combined damage in comparison with other experimental groups. This suggests that the compensated stage of heart failure was not present in this model. The more pronounced progression of cardiac dysfunction due to the concomitant renal dysfunction has also not been observed. However; it must be remembered that the renal damage in this model was only mild, and there was a short period of time between induction of renal and cardiac damage. This might have resulted in limited effects of renal dysfunction on heart tissue and its function.

The same group studied cardiorenal interaction in a combined model with a more severe renal dysfunction, introduced by means of SNX and 2 weeks later followed by coronary ligation [57]. In this model, the SNX induced proteinuria, decreased the creatinine clearance and increased histological changes, such as focal glomerulosclerosis, fibrosis, and mesangial matrix expansion. Introduction of the cardiac dysfunction on top of existing renal dysfunction did not influence the levels of proteinuria nor the histological changes, but led to decrease in creatinine clearance and RBF. With regard to cardiac function, combination of renal and cardiac function did not yield additional effects, different from the effects of SNX or MI alone. Animals with combined organ damage had high blood pressure, similar to the animals with renal damage alone, and decreased contractility and relaxation comparable with the animals that underwent coronary ligation only. Also, decrease in capillary density was the same as in the MI alone group, showing no effect of

preexisting renal dysfunction on myocardium. This is somewhat in contrast to literature findings that showed histological changes in the myocardium of the subnephrectomized animals [58, 59]. However, again short period of time for the development of renal dysfunction and lack of signs of severe cardiac failure have to be considered. This model has also been used in studies on the effects of RAAS inhibition on the cardiorenal interaction. It has been shown that treatment with lisinopril for 6 weeks prevented further histological changes in the kidney tissue or even reversed some of them. Moreover, it restored the decreased RBF and creatinine clearance levels. It also increased capillary density and reversed the cardiac hypertrophy, proving the beneficial effects of this treatment and usefulness of this model in studies on RAAS activation in the cardiorenal syndrome. A combined model of MI and SNX has been used by Dikow et al. [62] to study cardiac histological changes as an underlying mechanism of progression of cardiac disease in cardiorenal interaction. In this study with transient coronary ligation followed by reperfusion, it has been shown that myocardium of the rats with renal dysfunction is more susceptible to ischemia injury, which may explain the higher death rate after MI in renal patients. Whereas the total unperfused area was not different between animals with and without renal dysfunction, which would be unlikely as it reflects the anatomy of the coronary vasculature in the rat, the infarcted area was significantly higher in uremic animals. This effect was also present in animals treated with antihypertensive treatment and low- and highsalt diet, which excludes the underlying confounding effects of hypertension, sympathetic overactivity, and salt retention. The same group analyzed also the myocardial remodeling in a model of more permanent MI [56]. They have shown more advanced left ventricular remodeling in a model with cardiorenal failure as documented by left ventricular hypertrophy, decreased capillary density and increased fibrosis. Moreover, these changes were accompanied by decrease in cardiac function as assessed by echocardiography. Interestingly, the presence of anemia has also been reported in the animals with combined organ damage. This could be of special interest, since anemia is being considered one of the cardiorenal connectors [33, 63] and beneficial effects of treatment for anemia have been shown in experimental studies. In MI heart failure model, erythropoietin has been shown to improve cardiac function and induce neovascularization [64]. On the other hand, in subnephrectomized rats, anemia seemed to have protective effect on kidney structure [65]; however, this effect has not been investigated in a model with concomitant heart failure. Therefore, this model could also help to understand the role of anemia in the cardiorenal syndrome.

Bongartz et al. [66] have recently developed a new animal model of combined cardiorenal failure. This model is based on the hypothesis that oxidative stress and the dysbalance between nitric oxide (NO) and ROS are one of the cardiorenal connectors [9,63]. The inhibition of NO synthase in rats has already been shown to cause hypertension, cardiac dysfunction, and glomerular damage [67, 68]. In the MI model, oxidative stress has been proven to decrease endothelial-dependent relaxation [50], whereas antioxidant therapy prevents cardiac structure alterations [69]. In the described model, renal dysfunction has been induced by means of SNX,

whereas cardiac dysfunction was a result of NOS inhibition with Nx-nitro-L-arginine (L-NNA). The combination of the above led to severe cardiac dysfunction with increased blood pressure, enddiastolic volume, and decreased ejection fraction. Signs of congestion, increased cardiac fibrosis, and myocyte hypertrophy accompanied the functional changes. With regard to the kidney function, the combination of SNX and L-NNA resulted in increased plasma urea and creatinine levels and proteinuria. However, only the latter was significantly higher than in animals with SNX alone. On the histological level, combined cardiorenal damage was characterized by increased FGS and tubulointerstitial injury. Interestingly, the above changes had permanent character and were not reversed even when treatment with L-NNA was stopped. This model proves the importance of oxidative stress in cardiorenal interaction and might serve as a promising tool for further investigation of cardiorenal connectors.

An alternative model, which does not require surgical intervention, is the model of adriamycin-induced renal damage. It has been shown that administration of adriamycin leads not only to deterioration of kidney function, but heart function is also affected [70]. Both cardiac and renal dysfunction resulting from adriamycin administration are characterized by many features observed in humans with cardiorenal failure—both on functional as well as on the histological level [71–73]. However, the dose required to induce the cardiac cardiomyopathy is much higher than the one resulting in severe kidney dysfunction. Therefore, the possibilities for investigation of dysfunction of both organs at the same time are limited. Moreover, it is important to mention that many of those changes are rather the effect of the toxicity of adriamycin than involvement of known cardiorenal connectors.

Discussion and future perspectives

In this review, we summarized the available data on animal models used in the studies on cardiorenal interaction. The choice of a proper model is crucial, because results obtained in experimental studies may contribute to better understanding of this important clinical syndrome and help in establishing new treatment strategies. At this moment, this translation is limited, because none of the models described above entirely reproduce the pathophysiology and characteristics of the cardiorenal syndrome observed in humans. Therefore, there is a need for better models of cardiorenal interaction that not only should mimic clinical characteristics of this syndrome by cardiac, renal, hemodynamic, and neurohumoral alterations, but also allow evaluation of the effectiveness of treatment. Such a model should consist of combined renal and cardiac injury characterized by progressive deterioration of function of both organs. With regard to the cardiac function, it should be characterized by systolic dysfunction confirmed by echocardiography or hemodynamic measurements, resulting in decrease in cardiac output and subsequent reduction in RBF. Also, increased end-diastolic pressure and venous congestion should be present in order to further investigate latest findings from clinical observations. On

the histological level, cardiac injury should be characterized by hypertrophy and fibrosis and preferably with cardiomyocyte/ capillary mismatch. With regard to renal injury, such a model should be characterized by a progressive decrease in renal function reflected in increased levels of creatinine and proteinuria or increased albumin excretion and decreased GFR/creatinine clearance. The presence of glomerulosclerosis and interstitial fibrosis should be documented. Important in developing a new model is to reduce the function of both organs to such a degree that leads to development of significant dysfunction as well as to provide enough time for its development. Both organ failures should also preferably develop gradually to allow studies on treatment for advanced stages as well as more preventive treatment strategies. This can help in drawing conclusions and give more insight into pathophysiology behind cardiorenal interaction on different stages of its development.

There are multiple ways of inducing cardiac [74, 75] and renal dysfunction [76] in rodents, which can be combined for the development of a new model of cardiorenal interaction. They all have their advantages and drawbacks, and none of them presents all the above-mentioned features of the ideal model. Most of the models require surgery intervention or administration of exogenous substances, which already reduces their similarity to the clinical situation. Furthermore, the surgical interventions, such as MI or reduction in kidney tissue, are performed on healthy organs in a situation where the regulatory mechanisms are not being activated. In humans, on the other hand, the failure is usually preceded by gradual loss of the function of chronically affected organs. The acute onset of the organ failure substrate is one of the common drawbacks that limit the clinical relevance of many popular models, such as transverse aortic constriction. An important issue in the use of animal models is also the severity of the induced organ failure. Moderate interventions result either in the development of mild organ dysfunction or the organ failure will develop only in a subset of the animals [48, 55, 75]. A more severe approach leads to the development of overt organ failure [48, 77], but results in high mortality rates [78] and more acute onset of the heart failure substrate. This limits the clinical relevance of such models. The usefulness of the models based on surgical interventions is also limited by the fact that they lead to significant reduction in remaining tissue mass, like in the SNX model or the MI model. This reduces the potential target and the effects of treatment, as well as possibilities for molecular analysis. Important to remember is also that the differences in response to surgical interventions have been reported between different genders [79] or animal strains [80, 81]. Above-described drawbacks of available animal models might question the possibility of designing one perfect animal model of the cardiorenal syndrome. However, it should be remembered that heart and renal failure are heterogeneous disorders that result from multiple underlying diseases and involve various regulatory mechanisms. Due to the complexity of the pathophysiology of heart and/or renal failure alone and in combination, its proper understanding might as well require the use of more than one animal model and the choice must be made depending on the research question. The heterogeneous character and origin of cardiorenal failure require also that all potential new models as well as the models

already known, including those described in this review, should be studied in more detail. Not only well-established cardiorenal connectors, but also new findings in the cardiorenal interaction, such as increased venous congestion, anemia, and tubular damage need further investigation and should be addressed in these models. Only then, the usefulness of these models in studies on the cardiorenal syndrome can be evaluated.

Interesting alternative for the models described above might be the use of genetic models of cardiac and renal dysfunction. One of the biggest advantages of such models is that they do not require surgery or pharmacological intervention for induction of the disease. The onset of the stimulus for organ failure is more gradual, which allows studies on progression of the disease and prevention therapy. Well-studied genetic models of heart failure are the spontaneously hypertensive rat (SHR) [82] and the Dahl salt-sensitive rat [83]. However, both strains require long time before they develop overt heart failure, which is their main drawback. The Munich Wistar Fromter (MWF) rats present many of the clinical characteristics of renal dysfunction [84]. However, again an extended period of time is required for the development of the renal failure. Moreover, some gender differences have been reported [85]. The genome of rodents has recently been well characterized, which provides new opportunities in finding a new better model for cardiorenal interaction. The ability of genetic manipulation offers multiple possibilities for establishing new valuable models. Knocking out or overexpression of specific genes or proteins as well as transgenesis may lead to the development of new models of organ failure [86–89]. This approach gives a good insight into role of a given gene or protein in the pathophysiology of the disease. Knock-out models can help also in understanding the pathophysiology of the diseases preceding the onset of organ failure, such as atherosclerosis [90]. However, the genetic manipulation does not allow control over the time of the occurrence or the level of intervention. Change in the expression of specific proteins may also influence their biological properties and function [91] as well as activate the compensatory mechanisms at very early stages.

Conclusion

There are only few animal models proposed for further studies on the cardiorenal syndrome. They all represent to some extent clinical characteristics of this syndrome and were used in experimental studies to better understand the pathophysiological connections between kidney and the heart. However, the heterogeneous character of heart and renal failure in humans limits the possibilities of complete reproduction of this syndrome in an animal model. Moreover, the induction of the organs failure in animals has usually an acute character, whereas heart and/or kidney dysfunction in humans usually develop over years. The recent developments in molecular techniques provide promising possibilities of developing a new animal model, which could be used as a tool for better understanding of the pathophysiology and treatment targets in the cardiorenal syndrome.

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Chapter 3

Increased sensitivity to cardiac ischemia in proteinuric rats is not attributed to adverse cardiac remodeling

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Submitted

Abstract

Background. Chronic renal dysfunction severely increases cardiovascular risk. Adverse cardiac remodelling is suggested to play a major role as predisposition for increased cardiac ischemic vulnerability. Aim of the present study is to examine cardiac sensitivity to acute ischemia/reperfusion damage in a rat model for renal dysfunction and to evaluate the role of adverse cardiac remodelling in this process.

Methods. Munich Wistar Fromter rats (MWF), developing spontaneous progressive renal dysfunction and mild hypertension, were compared to healthy Wistar control, and to non-proteinuric spontaneously hypertensive rats (SHR). In 12 weeks old MWF, renal dysfunction and mild hypertension were confirmed. Hearts were analyzed for adverse remodeling, including myocyte hypertrophy, capillary density and interstitial fibrosis, by histology. Sensitivity to cardiac ischemia/reperfusion damage was obtained from infarct size measured after 30 minutes coronary artery occlusion, followed by 24 h of reperfusion.

Results. Infarct size was higher in SHR (52±4%; p=0.16) and in MWF (56±3%; p=0.04), when compared to Wistar (45±4% of risk area). However, whereas SHR showed pronounced adverse cardiac remodelling, MWF displayed 29% (NS) larger left ventricular myocytes (vs 72% in SHR), without decreased capillary density (MWF +34%; SHR -13%) or interstitial fibrosis (MWF -16%; SHR +70%).

Conclusion. Data indicate that chronic renal dysfunction in MWF induces increased cardiac sensitivity to acute ischemia/reperfusion damage, as reflected by higher infarct sizes. Comparing results to SHR suggests that this increased susceptibility could not be attributed to hypertension or adverse cardiac remodeling.

Introduction

Impaired renal function is an important risk factor for cardiovascular disease [1-5] and its presence and severity is associated with worse outcome. In addition to factors associated with the kidney and circulation, including reduced renal perfusion, peripheral endothelial dysfunction, neurohumoral activation and hypertension [6-8], also cardiac factors could contribute to a reduced cardiac ischemic tolerance in renal dysfunction [9].These factors mainly include aspects of adverse cardiac remodeling: myocyte hypertrophy, cardiac capillary/myocyte mismatch [10,11] and loss of myocytes [1], as extensively reviewed by Tyralla and Amann [12], but also coronary endothelial dysfunction [13] and metabolic changes [14,15] may play a role. Data from animal models is scarce. Dikow and coworkers reported that rats, that are uremic due to acute subtotal nephrectomy, showed increased sensitivity to cardiac ischemia/reperfusion, which could not be attributed to hypertension, sympathetic overactivity or salt retention [9]. In the same model increased cardiac ischemic susceptibility was associated with abnormal cardiac energetics [14], while infusion of insulin and glucose could protect the heart from ischemia/reperfusion damage [15].

Since renal dysfunction in patients usually progresses slowly over time and even mild renal dysfunction is already associated with increased cardiovascular mortality and morbidity [16,17]. Munich Wistar Fromter rats (MWF) that spontaneously and slowly develop proteinuria with concomitantly focal glomerulosclerosis and mild hypertension with age [18], may mimic the clinical condition. Although literature extensively describes the renal abnormalities in this model [19], cardiovascular effects have hardly been investigated. We have previously shown accelerated cardiac but not renal dysfunction after chronic myocardial infarction [20]. Moreover, we have observed the endothelial dysfunction present in coronary- but not in mesenteric arteries [13], which was not observed in hypertensive non-proteinuric SHR rats, indicating a hypertension-independent effect.

Aim of the present study was to investigate cardiac sensitivity to acute ischemia/reperfusion damage in MWF rats in comparison to healthy Wistars and hypertensive SHR. Since adverse cardiac remodeling is suggested to present a predisposition for increased cardiac risk, its role in acute ischemia reperfusion damage was evaluated.The study reports on increases in infarct size, as measure of ischemic damage in rats with renal dysfunction, but without preexisting adverse cardiac remodeling.

Material and Methods

Animals

The study was performed in male rats from 3 different strains: Wistar (HsdCpb:WU) obtained from Harlan The Netherlands and Munich Wistar Fromter (MWF/ZtmHsd) and Spontaneously

Hypertensive Rats (SHR/NHsd) both obtained from Harlan USA. Animals were housed in groups under standard conditions at 12h light/dark cycle until they reached 12 weeks of age at the animal facilities of the University of Groningen. All animals were fed standard diet (standard rat chow, Hope Farms, Woerden, The Netherlands) and received food and water ad libitum during the study. All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Committee for Animal Experiments of the University of Groningen.

Experimental protocol

All rats were placed in metabolic cages in order to measure water and food intake and 24-hour urine collection. Subsequently, under isoflurane in O2 anesthesia, blood samples were withdrawn from the tail vein and plasma was processed. Plasma and urine samples were stored at -80 °C for later analysis. Rats were left one week to recover from this procedure.

Subgroups of 5-6 rats of each strain were used as a control and sacrificed to obtain baseline measurements of cardiac hemodynamics and to collect cardiac tissue for analysis of adverse cardiac remodeling as predisposition for cardiac vulnerability. The remaining rats (N=20 per strain) were subjected to 30-minute coronary artery occlusion (CAO) followed by 24 h of reperfusion [21,22]. This time point was chosen, because it is long enough to exclude effects on velocity of infarct development [22], but too short to exert effects on cardiac remodeling. The latter being important as we aimed to investigate adverse cardiac remodeling as predisposition for, rather than as consequence of, altered sensitivity to acute ischemic damage. Blood samples were collected from the abdominal vena cava and plasma was processed and stored for later analysis. The heart was dissected for measurement of area at risk and infarct size. The method for accurate measurement of infarct size does not allow cardiac tissue to be used for histology and/or molecular analysis. Renal tissues were collected, weighted and processed for further analysis.

Urine measurements

Total urinary protein level was determined using endpoint measurement with TCA precipitation (Nephelometer analyzer II, Dade Behring, Marburg, Germany). Urine creatinine was determined using a standard kit (Roche Diagnostics, Basel, Switzerland). In addition to urinary protein excretion as a representation of renal function, creatinine clearance, as an estimate for glomerular filtration rate, was calculated from urinary and plasma creatinine levels. creatinine clearance = (urine creatinine x urine flow) / (plasma creatinine x body weight) and presented as mL/min/kg body weight.

Blood measurements

Measurements of plasma creatinine, electrolytes levels and hematocrit were performed using iSTAT handheld analyzer (ABBOTT) and appropriate cartridges (Creatinine; EG7+).

Catecholamines measurements were performed in plasma with glutathione added as a preservative as described before [23], brain natriuretic peptide (BNP) was measured with BNP-45 EIA kit (Phoenix Pharmaceuticals, Inc) (with protease inhibitor aprotinin added [24]) and Plasma Renin Activity (PRA) was determined by Gammacoat RIA kit (Diasorin, Minnesota, USA) and expressed as ngAngI/ml/h [25].

Cardiac and renal hemodynamic measurements

Control rats were anesthetized in 2,5% isoflurane and cardiac performance was measured by a pressure catheter (Micro-Tip 3French, Millar Instruments Inc., Houston,TX) inserted through the right carotid artery and advanced into the left ventricle. After a period of stabilization, left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP) and heart rate (HR) were recorded. The maximal rates of increase and decrease in left ventricular pressure (+dP/dtmax and –dP/dtmax) were measured as parameters of myocardial contractility and relaxation, respectively.

Then, the catheter was withdrawn into the aortic root and arterial blood pressure was measured. Subsequently, the catheter was inserted in the jugular vene and advanced to just above the heart. After stabilization, central venous pressure (CVP) was measured.

Ischemia/reperfusion

Under 2.5 % isoflurane anesthesia rats were subjected to experimental 30-minute coronary artery occlusion as described before [26]. Briefly, after left side intercostal thoracotomy, the pericardium was opened and the left descending coronary artery was occluded with a 6-0 silk suture, 1-2 millimeters from its origin. After 30 minutes the suture was opened for reperfusion and subsequently the thorax was closed. Animals were allowed to recover. Occlusion and reperfusion were visually verified by appearance and disappearance of myocardial cyanosis. As body temperature affects sensitivity to ischemia/reperfusion [27], rectal temperature was continuously measured with an electronic thermometer and was maintained in the range 36.5 – 37.5 C by either heating pad or cold-water filled packages. The adequacy of this procedure was verified before [26]. Rats that fibrillated during ischemia or reperfusion were allowed to complete the protocol if conversion to sinus rhythm occurred spontaneously or resuscitation by gently thumping on heart or thorax was successful within 5 minutes after onset of arrhythmia. Animals were allowed to recover and 24 hours later the rats were re-anesthetized to collect hearts for measurement of infarct size.

Organ Isolation

Hearts were rapidly excised and placed in ice-cold saline for diastolic arrest. Hearts of ischemia/reperfusion rats were processed for evaluation of ischemic damage. From the hearts of rats that were sacrificed before ischemia/reperfusion to obtain baseline measurements, a mid-ventricular slice has been processed for histochemical analysis.

Both kidneys were excised, weighted and fixated in 2% paraformaldehyde. The fixated tissues were then processed for paraffin embedding according to standard procedures. Lungs were excised and weighted.

Measurement of area at risk and infarcted area

Measurements of area at risk and infarcted area were performed as described before in details [22]. Briefly, the cooled heart was mounted to a modified Langendorff apparatus and perfused with ice-cold saline to wash out blood. After closing the ligature, heart was perfused with Trypan Blue to delineate normally perfused myocardium from the non-perfused area at risk (AR). The heart was then frozen in -80°C for 10 minutes and cut into slices of approximately 1mm from apex to base. Each slice was divided into right ventricle, normally perfused left ventricle and AR. The AR was subsequently incubated in Nitro-Blue-Tetrazolium to distinguish between irreversible damaged (unstained) and stained viable tissue. Unstained infarcted tissue was then isolated and all different areas of LV were dried and weighted separately. Infarct size was expressed as a percentage of total LV as well as percentage of AR.

Histology

Hearts of the rats that were sacrificed before ischemia/reperfusion were processed for histological analysis. For that, at mid-ventricular level, a transversely cut slice was fixated in 3.6% phosphate buffered formaldehyde for at least 24 h, slices were dehydrated and paraffin embedded. Deparaffinized 5 µm sections were either stained with Gomori's silver staining in order to visualize individual myocytes or with Lectin GSI to visualize capillaries or with Sirius Red to visualize interstitial collagen. Methods as well as the measurements of myocyte size, capillary density and interstitial fibrosis have been described before [28]. Briefly, as concentric rather than eccentric left ventricular hypertrophy is associated with sensitivity to ischemia [29], myocyte size was measured as cross-sectional area of transversally cut myocytes at the level of the nucleus, in the free wall of the left ventricle. In the same area, the number of capillary profiles were counted and expressed per tissue area. Interstitial fibrosis was obtained as the volume fraction of the Sirius Red positive area.

Kidneys were stained with PAS staining and scored on presence and degree of focal glomerulosclerosis (FGS) as described before [30]. Briefly, the degree of mesengial matrix expansion (MME) and FGS were assessed in 50 glomeruli by scoring semi quantitatively from 0 to 4, referring to number of quadrants affected. FGS was scored positive when MME and adhesion to Bowman's capsule were present in the same quadrant. FGS score was expressed in arbitrary units (AU).

Statistical analysis

Data are presented as mean ± standard error of the mean (S.E.M.). Parameters were compared using one-way analysis of variances (ANOVA) with Dunnett's test (against Wistar

group as control) for post-hoc analysis for multiple comparisons. In case data was not normally distributed, a non-parametric Kruskal-Wallis test followed by a Mann–Whitney U test with correction for multiple comparisons was used. Differences were considered significant at the level of 0.05.

Results

Primary renal dysfunction

General characteristics of the experimental groups are presented in table 1. At the same age, MWF as well as SHR had significantly lower body weights than Wistar, which could not be attributed to lower food- or water intake. Plasma electrolytes levels were not different among the groups. MWF, but not SHR, had a significantly lower plasma hematocrit. Renal dysfunction in MWF was confirmed by significantly increased urinary protein excretion. However, neither absolute plasma creatinine levels nor creatinine clearance were different between MWF and Wistar (table 1).

Renal histology was analyzed in order to confirm that observed renal functional changes had

Table 1. *Baseline characteristics of ischemia/reperfusion animals.. Data are presented as mean ±SEM. SHR: Spontaneously Hypertensive Rats; MWF: Munich Wistar Fromter rats; * p<0,05 vs Wistar*

	Wistar	SHR	MWF
N	17	19	19
Body weight (g)	394±8	300±5*	274±7*
Food intake (g/24h)	11±1	7±1	13±1
Water intake (ml/24h)	21±2	19±1	23±1
Urine production (ml/24h)	14±1	11±1	12±1
Plasma Na (mmol/L)	137±1	137±1	136±1
Plasma K (mmol/L)	4.6±0.1	4.3±0.1	4.8±0.2
Plasma Ca (mmol/L)	1.19±0.06	1.07±0.07	1.07±0.06
Hematocrite (%)	43±0	44±0	40±1*
Urinary protein excretion (mg/24h)	31±2	32±1	80±13*
Plasma creatinine (mmol/L)	32±2	27±1*	31±1
Urinary creatinine (mmol/L)	11.6±1.1	8.5±0.7	9.3±0.6
Creatinine clearance (ml/min/kg)	8.2±0.5	7.6±0.4	9.2±0.5

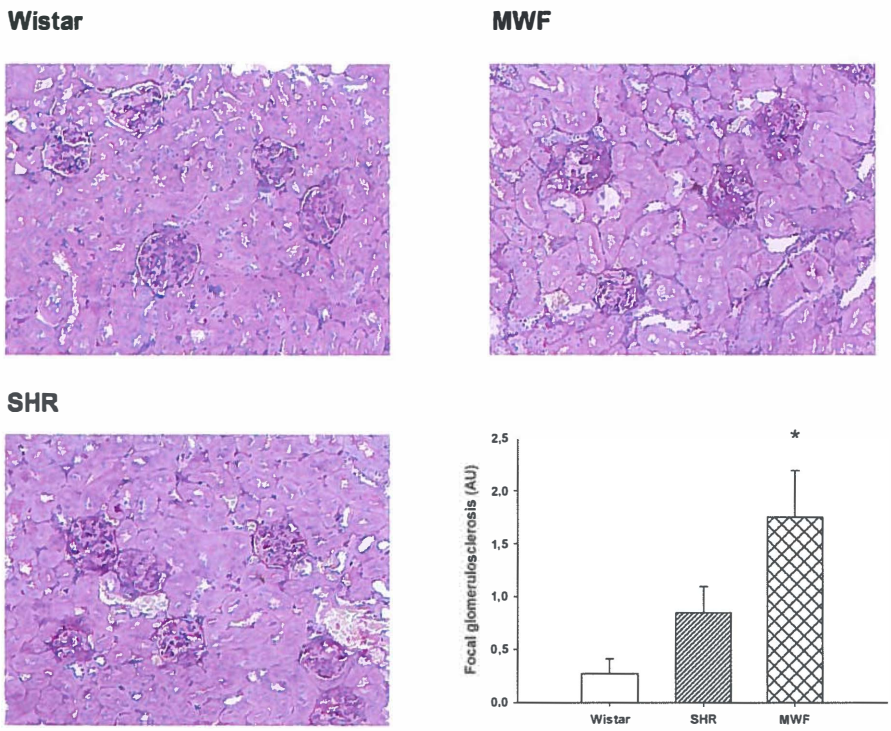

*Figure 1. Representative photographs of glomeruli in Wistar, SHR and MWF rats as well as results of scoring focal glomerular sclerosis (FGS). SHR: Spontaneously Hypertensive Rats; MWF: Munich Wistar Fromter rats. * p<0.05 vs Wistar*

lead to, or resulted from, a structural substrate. Figure 1 shows representative photographs of glomerular changes in the different rat strains, as well as the results of the quantification of these changes; MWF, but not SHR, displayed significantly higher scores of focal glomerulosclerosis (FGS). FGS was significantly correlated with urinary protein excretion in MWF (r^2=0.374; p<0.01) but not in SHR or Wistar. There was no correlation between FGS and creatinine clearance in any of the groups.

Cardiac predispositions

Hemodynamics

Baseline measurements revealed mild hypertension in MWF and pronounced hypertension in SHR, without differences in heart rate or central venous pressure (CVP) (table 2). Cardiac function parameters are presented in figure 2, showing significantly increased LVSP in MWF and SHR, but with substantially higher values in SHR, without effects on LVEDP, +dP/dt or –dP/dt. Heart weight was significantly increased in both MWF and SHR; lung weight only in MWF. Kidney weight was highly preserved in the rat strains (table 3).

*Table 2. Baseline characteristics of control animals. Data are presented as mean ±SEM. MAP: mean arterial pressure; HR: heart rate; CVP: central venous pressure; SHR: Spontaneously Hypertensive Rats; MWF: Munich Wistar Fromter rats; * p<0.05 vs Wistar;*

	Wistar	SHR	MWF
N	5	6	5
MAP (mmHg)	89±4	136±6*	102±3*
HR (beats/min)	364±13	354±17	359±18
CVP (mmHg)	1.2±1.8	-1.7±1.7	2.1±1.7
Heart weight/body weight (g/kg)	3.2±0.2	4.5±0.1*	4.5±0.5*
Lung weight/body weight (g/kg)	3.3±0.1	3.7±0.1	4.8±0.1*

*Table 3. Organ characteristics at termination; Data are presented as mean±SEM; BW; body weight; LV left ventricle; RV right ventricle SHR: Spontaneously Hypertensive Rats; MWF: Munich Wistar Fromter rats. * p<0.05 vs Wistar;*

	Wistar	SHR	MWF
N	17	19	19
Ventricular weight/body weight (g/kg)	2.5±0.1	3.7±0.1*	3.0±0.1*
LV weight/body weight (g/kg)	2.2±0.1	3.2±0.1*	2.6±0.1*
RV weight/body weight (g/kg)	0.49±0.02	0.71±0.04*	0.57±0.02*
Lung weight/body weight (g/kg)	4.5±0.1	5.0±0.1*	4.8±0.1
Left kidney weight/body weight (g/kg)	3.6±0.1	3.5±0.1	3.6±0.1

Cardiac remodeling

Adverse cardiac remodeling as predisposition for increased sensitivity to ischemia was analyzed in control rats. Representative photographs of histological sections stained for myocytes, capillaries and interstitial collagen are shown in figure 3A. In general, tissue density was not different among the strains, ranging from 91 to 94 %. Myocyte hypertrophy was indicated by a mild and non-significant increase in cross-sectional area of myocytes in MWF (NS), but a significant increase in SHR ($p<0.05$). Staining of tissue area in MWF compared to Wistar, resulting in significantly higher capillary/myocyte ratios in MWF than in Wistar (1.92±0.28 vs 1.14±0.19; $p=0.05$). Volume percentage of collagen was similar in MWF and Wistar. In contrast, SHR did show increased interstitial fibrosis. Quantification of these observations is summarized in figure 3B.

Ischemia/reperfusion

Fifty-five out of 59 rats (one MWF rat was excluded due to lack of overt proteinuria and

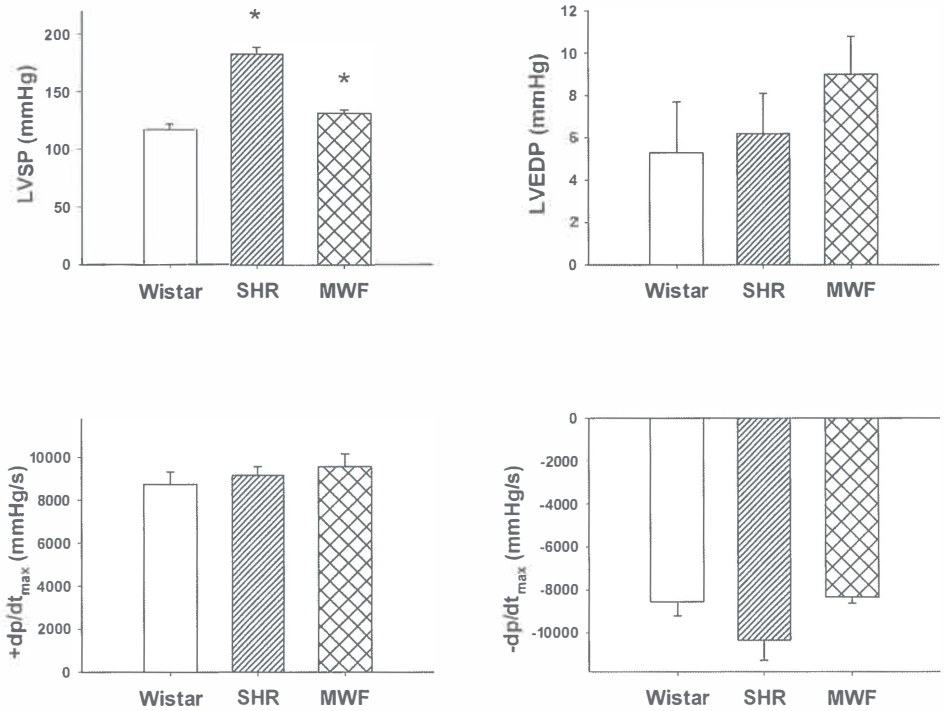

Figure 2. Cardiac function in control rats; presented by left ventricular systolic pressure (LVSP), left ventricular enddiastolic pressure (LVEDP), velocity of contraction (+dP/dtmax), velocity of relaxation (-dP/dtmax) for the 3 groups. SHR: Spontaneously Hypertensive Rats; MWF: Munich Wistar Fromter rats. p<0.05 vs Wistar;*

substantially lower body weight) survived the ischemia/reperfusion protocol; 17 of Wistar, 19 of MWF and 19 of SHR. Death usually occurred within the first hour after ischemia.

Occlusion of the left descending coronary artery resulted in a risk area of about 45% of the left ventricle. On average, risk area was significantly higher in both MWF (47±2%) and SHR (49±3%) compared to Wistar (39±3%). Infarct size, expressed as a percentage of total left ventricle (LV) was significantly increased in MWF (28±2%) and SHR (26±2%) in comparison to Wistar (17±2%)(figure 4A). Since infarct size has been found linearly related to risk area [21], infarct size expressed as percentage of the individual capillaries revealed even higher number of capillaries per risk area, was used to present sensitivity to ischemia/reperfusion. A significantly higher infarct to risk area in comparison to Wistars (45±4%) was found in MWF (56±3%; $p<0.04$), whereas only a trend was observed in SHR (52±4%, $p=0.16$) (figure 4B). There was no significant correlation found between any of the renal parameters and infarct size.

At termination, increased ventricular weight corrected for body weight was present in both MWF en SHR, and both the left and the right ventricle had contributed to this increased

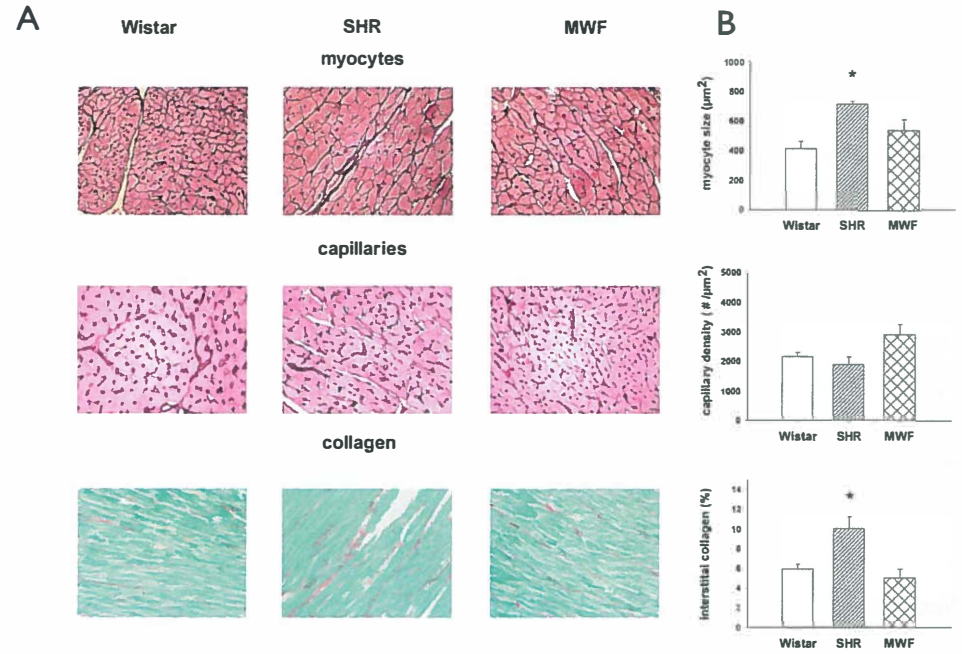

Figure 3.A: *Representative photographs of Gomori staining to show individual myocytes (top); Lectin staining to visualize capillaries (mid) and Sirius Red staining to identify cardiac fibrosis (bottom) in MWF, SHR and Wistar.* ***B:*** *Accordingly, quantification of the observations in the 3 strains. SHR: Spontaneously Hypertensive Rats; MWF: Munich Wistar Fromter rats. * p<0.05 vs Wistar;*

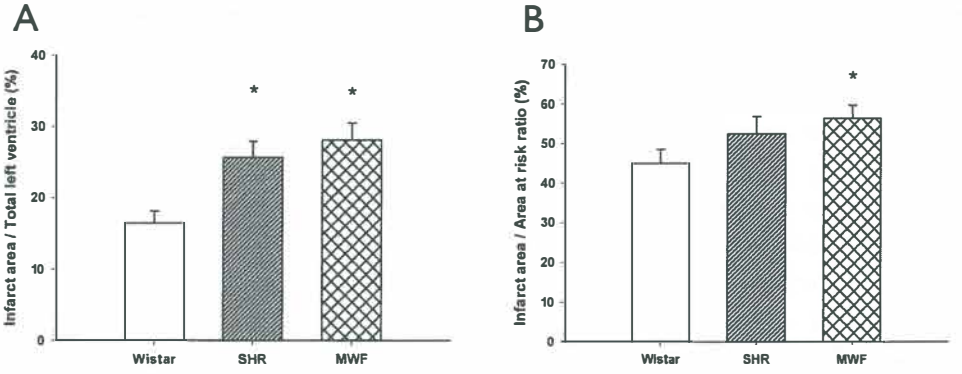

Figure 4. *Infarct size presented as* ***A:*** *percentage of total LV and* ***B:*** *percentage of area at risk in Wistar, SHR and MWF rats. SHR: Spontaneously Hypertensive Rats; MWF: Munich Wistar Fromter rats. *: significantly different from Wistar (p<0.05)*

weight in both strains (table 3). Similar dry weight/wet weight ratio in all groups (0.21-0.22) excluded differences in cardiac edema.

Neurohumoral factors

At baseline, no differences in catecholamines and PRA levels were found between groups, whereas BNP levels were lower in MWF in comparison to other strains (table 4). After ischemia/reperfusion, PRA was significantly lower in MWF than in Wistar rats. PRA after ischemia/reperfusion in MWF was significantly correlated with infarct size (r^2=0.315; p=0.03), but not with renal function parameters. Regarding PRA, SHR did not differ from Wistar, nor could a correlation with infarct size be demonstrated.

Noradrenaline and adrenaline levels after ischemia/reperfusion were similarly increased in all strains in comparison to respective baseline values, with the highest values of noradrenaline in SHR. Circulating dopamine levels were not different at baseline between groups and no response to ischemia/reperfusion was observed (data not shown).

BNP levels after ischemia reperfusion were similar in all strains. No correlation with infarct size was observed. BNP levels were neither correlated with catecholamines nor with PRA, neither at baseline nor after ischemia/reperfusion.

Discussion

In the present study we investigated the role of adverse cardiac remodeling as predisposition for increased sensitivity to cardiac ischemia in an animal model (MWF) of spontaneous progressive renal dysfunction. We showed increased sensitivity to ischemia in MWF rats, which could not be attributed to hypertension or adverse cardiac remodelling. These findings are in accordance with the literature [9], and suggest alternative mechanisms to play a role in increased heart ischemic sensitivity in renal dysfunction.

Increased sensitivity to ischemia

Sensitivity to cardiac ischemia was investigated by the amount of irreversible damaged tissue after acute ischemia/reperfusion. Area at risk appeared to consist of 39-49% of the left ventricle. The observed differences in area at risk between the groups may be attributed to strain-related differences in coronary anatomy since rats are not known to present with coronary collaterals. The relationship between area at risk and infarct area is found linear at the range of 25-75% risk area [21], justifying expression of infarct size per risk area as a measure of cardiac damage. Accordingly, the significant increase of infarct size in MWF represents increased sensitivity to acute cardiac ischemia reperfusion damage in rats with renal dysfunction.

Underlying mechanisms

Cardiac related

It has been shown that changes in cardiac function and structure can lead to increased ischemic sensitivity [10-15]. Except for a mild increase in left ventricular systolic pressure, no significant

*Table 4. Neurohumoral levels in plasma of the experimental groups at baseline and after ischemia reperfusion; Data are presented as median (25-75 quartile); PRA; plasma renin activity; BNP; brain natriuretic peptide; * p<0,05 vs Wistar;*

	Wistar	SHR	MWF
Baseline			
PRA (ngAngl/ml/min)	6.6 (5.6-9.1)	8.1 (6.6-11.8)	5.0 (4.0-7.0)
Noradrenaline (pg/ml)	411 (312-657)	479 (184-597)	445 (237-635)
Adrenaline (pg/ml)	55.5 (10.0-108.0)	37.0 (10-114)	16.0 (10.0-51.0)
BNP (ng/ml)	3.3 (2.2-3.7)	3.6 (2.8-4.7)	1.8 (1.4-2.4) *
After ischemia/reperfusion			
PRA (ngAngl/ml/min)	40.0 (34.3-61.8)	50.0 (38.0-59.0)	24.5 (19.2-36.4) *
Noradrenaline (pg/ml)	822 (631-1079)	849 (584-1510)	716 (482-838)
Adrenaline (pg/ml)	367 (160-705)	732 (41-1327)	399 (141-1053)
BNP (ng/ml)	2.8 (2.4-4.1)	4.8(2.8-5.9)	3.4 (3.0-4.8)

functional cardiac parameters have been observed that could explain the increased cardiac sensitivity to acute ischemia/reperfusion in MWF.

Myocytes: In MWF, cardiac hypertrophy was indicated from increased ventricular weight to body weight ratio. Left ventricular concentric hypertrophy by itself is associated with increased ischemic sensitivity [19] and was supported at cellular level by 29% increase in myocyte cross-sectional area in MWF. However, higher blood pressure and a more pronounced increase in ventricular weight in SHR did not lead to higher sensitivity to ischemia. This indicates that high blood pressure-induced left ventricular myocyte hypertrophy may not be the causal factor for the increased acute ischemic susceptibility in proteinuric rats. Increased lung weight and right ventricular weight may suggest that pulmonary congestion and right ventricular dysfunction might have contributed [6,20].

Alternatively, metabolic changes could play a role in increased ischemia sensitivity in rats with renal dysfunction. Studies on uremic rats showed increased ischemic sensitivity [9], which could be prevented by infusion of insulin and glucose [15]. Hearts are characterized by lower phosphocreatinin levels at normal creatinin and ATP levels [13,14]. Finally, alterations in Ca-handling [14,32], as well as altered phosphorilation of contractile proteins [33], shown to contribute to cardiac (diastolic) dysfunction, could have contributed.

Vasculature: Pressure overload-induced hypertrophy is usually associated with decreased capillary density, as microvascular adaptation is lagging behind myocyte growth [34]. In addition, macrovascular (resistance sized arteries) deficiency [35] may contribute, as coronary reserve is found reduced [36-38]. In the present study, hearts of MWF did not display lower capillary density and capillary/myocyte ratio was even higher in MWF than in Wistar. This is in contrast to subtotally nephrectomized rats [39], in which increased cardiac ischemic sensitivity was

attributed to reduced capillary density [9]. Since cardiac perfusion is determined at resistance arteriolar level rather than at capillary level, and in our previous study in 25 weeks old MWF maximal coronary capacity was not reduced compared to control [40], structural vascular deficiency seems to be ruled out as underlying mechanism.
However, functional vascular changes still may interfere with cardiac responses to ischemia [12]. Impaired endothelial function in aortic rings from MWF [20,40], but not from SHR or Wistar, supports functional vascular changes in MWF. A previous study from our group showed that in MWF, but not in SHR, endothelial function was impaired in coronary, but not mesenteric microvessels [13]. Hence, impaired endothelial function in the coronary circulation, could provide an explanation for the increased sensitivity to ischemia in MWF.
Connective tissue: Overall tissue density was not altered in MWF, indicating no increased oxygen diffusion distance through increased interstitial space. Also collagen content of the left ventricle, which could increase ventricular stiffness and hamper ventricular relaxation and cardiac perfusion, was not increased in MWF. In summary, since all aspects of adverse cardiac remodeling are either absent or less pronounced in MWF compared to SHR, the observed higher vulnerability to acute ischemic in MWF may not be explained by adverse cardiac remodeling. Moreover, the observation that cardiac remodeling in MWF does not match with studies in the 5/6 nephrectomy rat model [9] indicates that at different stages of renal dysfunction different mechanisms may play role in increased ischemic sensitivity. The lack of structural changes, however, does not exclude a role for functional alterations in vasculatures and/or myocytes.

Neurohumoral related

PRA, catecholamines and BNP have been measured to examine baseline neurohumoral activation as predisposition for increased cardiac ischemic sensitivity. BNP is mainly released from ventricular myocytes in reaction to stress or tension, and acts as an antagonist of the renin-angiotensin aldosterone and sympathetic nervous system to protect the body from volume overload [26]. Higher blood pressure, left ventricular hypertrophy, impaired coronary endothelial dependent relaxation [14] and increased circulating levels of cyclooxygenase products [28] may have contributed to this increased ventricular stress.
Peripheral endothelial dysfunction, observed in MWF but not in SHR or Wistar, is associated with altered cyclooxygenase metabolism [40]. Similar to the impaired endothelial function in the coronary circulation, these changes in peripheral vessels may serve as a marker for ischemic vulnerability in MWF.

Kidney related

In MWF, renal dysfunction was evidenced by increased urinary protein excretion and focal glomerulosclerosis, without differences in creatinine clearance, which represents either a less severe renal dysfunction than in uremic rats or a different type of renal dysfunction [41].

Even MWF rats twice as old as in the present study with advanced proteinuria and focal glomerulosclerosis, did not show reduced creatinine clearance [40]. Although in the present study the renal dysfunction was the primary factor to investigate, regarding its effect on sensitivity to ischemia, no correlation was found between renal parameters and infarct size. The relatively mild renal dysfunction may exclude a number of factors that have an impact on outcome in more advanced renal failure, such as metabolic acidosis, hyperkalemia, hyperparathyroidism and alterations in calcium homeostasis [9].

Finally, the consistent reduction in hematocrit levels in MWF and in subtotally nephrectomised rats [9], but not in SHR, is well known in chronic kidney disease [42,43]. Reduced hematocrit and lower number of red blood cells (data not shown) may account for impaired oxygenation during reperfusion, and hence limitation of repair. In addition to a possible hemodiluting effect of fluid retention, erythropoietin resistance is well known in renal patients. Moreover, recently some of these factors, including inflammation, a prothrombotic milieu, and anemia, are explicitly mentioned as "novel" risk factors in cardiorenal disease [44]

Study limitations

Because of the invasive character, baseline hemodynamics could not be measured in the same animals that underwent cardiac ischemia. Therefore, to examine cardiac and peripheral hemodynamics as predisposition for increased ischemic vulnerability, measurements were performed in separate rats. Secondly, because of the cardio-depressive effects of the ischemia/reperfusion protocol itself, cardiac function measurements after 24 h of reperfusion (although obtained) did not provide reliable results, and were hence not included in the paper.

Thirdly, we measured neurohumoral factors in plasma before and after ischemia reperfusion. The values after ischemia/reperfusion include effects of ischemia reperfusion as well as effects of the experimental procedure: chest surgery.

Finally, as blood sampling was not at the exact same time as urine collection, and measurements of creatinine used different techniques for urine and plasma, comparison between experimental groups remain valid, but absolute data for creatinine clearance may deviate.

Conclusion

Aim of the present study was to investigate increased cardiac ischemic sensitivity in a model of spontaneous progressive renal disease, MWF rats, and the role of preexisting adverse cardiac remodeling in this process. Our data indicate that MWF are more sensitive to cardiac ischemia. Comparing data from MWF and SHR, however, seems to exclude adverse cardiac remodeling as underlying mechanism. Vascular endothelial dysfunction, altered neurohumoral responsiveness and anemia may provide alternative explanations for the increased sensitivity

to ischemia/reperfusion damage seen in MWF hearts.

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Chapter 4

Increased cardiovascular risk in rats with primary renal dysfunction; mediating role for vascular endothelial function

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Abstract

Primary chronic kidney disease is associated with high cardiovascular risk. However, the exact mechanisms behind this cardiorenal interaction remain unclear. We investigated the interaction between heart and kidneys in novel animal model for cardiorenal interaction. Normal Wistar rats and Munich Wistar Fromter rats, spontaneously developing renal dysfunction, were subjected to experimental myocardial infarction to induce cardiac dysfunction (CD) and combined cardiorenal dysfunction (CRD), respectively (N = 5–10). Twelve weeks later, cardiac- and renal parameters were evaluated. Cardiac, but not renal dysfunction was exaggerated in CRD. Accelerated cardiac dysfunction in CRD was indicated by decreased cardiac output (CD 109 ± 10 vs. CRD 79 ± 8 ml/min), diastolic dysfunction (E/e') (CD 26 ± 2 vs. CRD 50 ± 5) and left ventricular overload (LVEDP CD 10.8 ± 2.8 vs. CRD 21.6 ± 1.7 mmHg). Congestion in CRD was confirmed by increased lung and atrial weights, as well as exaggerated right ventricular hypertrophy. Absence of accelerated renal dysfunction, measured by increased proteinuria, was supported by absence of additional focal glomerulosclerosis or further decline of renal blood flow in CRD. Only advanced peripheral endothelial dysfunction, as found in CRD, appeared to correlate with both renal and cardiac dysfunction parameters. Thus, proteinuric rats with myocardial infarction showed accelerated cardiac but not renal dysfunction. As parameters mimic the cardiorenal syndrome, these rats may provide a clinically relevant model to study increased cardiovascular risk due to renal dysfunction. Peripheral endothelial dysfunction was the only parameter that correlated with both renal and cardiac dysfunction, which may indicate a mediating role in cardiorenal interaction.

Introduction

Even mild renal dysfunction substantially increases cardiovascular risk [12, 20, 28]. Moreover, many patients with heart failure suffer from concomitant renal dysfunction [19], indicating a strong cardiorenal interaction. Although well recognized, the pathophysiology of this interaction is largely unclear. Patients characterized by a condition of primary chronic kidney disease [36] are at an extremely high cardiovascular risk; 10–20-fold increased risk of cardiac death; the 2 year mortality rate after myocardial infarction is increased up to 50%, compared to 10 year mortality in the general population of 25% [17]. These findings have revived interest in the interrelation between heart and kidneys. To investigate the interaction between heart and kidneys in this high-risk group of patients, different animal models have been used so far [41]. Most studies include rats with either unilateral [48] or 5/6 nephrectomy [9, 55] to induce (a predisposition for) renal dysfunction, that were subjected to chronic experimental heart failure. Although studies have presented accelerated renal dysfunction [48], in neither study accelerated cardiac dysfunction has been observed. Only one study reported worsening of cardiac dysfunction and exaggerated cardiac remodeling, but this could well be attributed to the larger infarcts in the cardiorenal group [10]. As all models have their advantages and disadvantages, the acute character of the nephrectomy in the above models and the little amount of renal tissue left for therapeutic intervention, provides a clear disadvantage in the translation of the results to the human cardiorenal syndrome. We aimed to study cardiorenal interaction in an alternative animal model, better mimicking the clinically high-risk condition; rats that spontaneously develop slowly progressive renal dysfunction, subjected to experimental heart failure. Parameters of renal as well as cardiac dysfunction were recorded and correlated to study interaction between heart and kidneys. As heart and kidneys are connected by vasculature, and endothelial function is reported to predict outcome in cardiac as well as renal dysfunction, vascular endothelial dysfunction was studied as potential mechanism underlying cardiorenal interaction.

Methods

Animals

The study was performed in male Munich Wistar Fromter rats (MWF/ZtmHsd) and age-matched Wistar rats (HsdCpb:WU), as their genetic background (Harlan, The Netherlands/ USA). Animals were housed in groups under standard conditions at 12 h light/dark cycle at the animal facilities of the University of Groningen. All animals were fed standard diet (standard rat chow, Hope Farms, Woerden, The Netherlands) and received food and water ad libitum. All experiments were conducted in accordance with the NIK Guide for the Care and Use of Laboratory Animals and were approved by the Committee for Animal Experiments of the

University of Groningen.

Experimental protocol

At 12 weeks of age, all rats were placed in metabolic cages for measurements of water and food intake and 24 hour urine collection. Subsequently, under 2.5% isoflurane anaesthesia blood samples (±1.5 ml) were withdrawn from the tail vein. Urine and plasma samples were processed and stored at -80°C for later analyses. Subgroups of Wistar and MWF rats (n = 5 each) were sacrificed prematurely to obtain baseline measurements of cardiac function and to collect cardiac and renal tissue for further analysis. The remaining animals were allowed 1 week recovery before they were subjected to permanent coronary artery occlusion or sham surgery as described before [52]. Five rats of each group (ECHO apparatus became available half way through the study) underwent echocardiography at baseline and at 3, 7 and 10–11 weeks after surgery to evaluate the time course of cardiac function. At the same time points, plasma and urine were collected from all rats (metabolic cages) to examine the time course of renal (dys)function. Twelve weeks after surgery, measurements of left ventricular function and cardiac and renal hemodynamics were obtained invasively. This time point was chosen because of anticipated endothelial dysfunction in CD animals [13]. Blood samples were collected from the tail vein and processed for further analyses. The heart was dissected, weighted and cooled in ice-cold saline for diastolic arrest. A mid-ventricular slice was processed for histological analysis. The remaining tissue was separated in right ventricle, interventricular septum, viable- and infarcted left ventricle, frozen in liquid nitrogen and stored at -80°C.

Lung tissue was weighted. Kidneys were dissected, weighted and processed for histological analysis.

The thoracic aorta was dissected and cut in rings for in vitro endothelial function analysis [44], as well as structural analysis.

Echocardiography

Echocardiographical measurements were obtained with the use of a Vivid 7 (GE Healthcare) equipped with a 10 MHz transducer. Under isoflurane anaesthesia (2.5%), rats were placed on a heating pad to keep body temperature at 37°C. Images in parasternal long-axis, short-axis and fourchamber apical view were obtained. The ejection fraction (EF) was calculated using the Teichholz method and cardiac output (CO) was estimated from the aortic flow profile and the diameter at valvular levels as follows: CO = aortic valve area times aortic velocity time integral times heart rate, as described before [45]. Mitral inflow measurement of early filling velocity (E) was obtained from an apical four-chamber view using pulsed Doppler with the sample placed at the tips of mitral leaflets. Tissue Doppler imaging measurement of mitral valve septal annular velocity (e') was also obtained from the four-chamber apical view. All calculated parameters have been presented as the mean of five consecutive beats to avoid beat-to-beat variation. As echocardiography could have been obtained only in subgroups, results should be

regarded as indication, rather than as group evaluation with statistical analysis.

Blood and urine measurements

After urine collection, blood samples were obtained under isoflurane anaesthesia. Measurements of plasma creatinine, electrolytes, haemoglobin, and haematocrit levels were performed with an iSTAT handheld analyser (ABBOTT) and appropriate cartridges (Creatinine; EG7+). Standard blood count was performed in whole blood samples. For that, whole blood sampled from the tail vein was transported immediately to the hospital. Remaining blood was processed and plasma was stored at -80°C for later analyses.

PRA was determined by Gammacoat RIA kit (Diasorin, Minnesota, USA) according to the manufacturer's instructions and expressed as ngAngI/ml/h. BNP was measured with the use of BNP-45 EIA kit (Phoenix Pharmaceuticals, Inc). Total urinary protein levels were determined using TCA precipitation measurement (Nephelometer analyzer II, Dade Behring, Marburg, Germany). All measurements were performed according to the manufacturer's instructions.

Hemodynamics and cardiac function

Under isoflurane anaesthesia, hemodynamics and cardiac performance were measured using a pressure transducer catheter (Micro-Tip 3French, Millar Instruments Inc., Houston, TX) inserted through the right carotid artery. The catheter was positioned in the aortic root and mean arterial blood pressure (MAP) and heart rate (HR) were measured. The catheter was advanced into the left ventricle, and left ventricular systolic pressure (LVSP) and left ventricular end-diastolic pressure (LVEDP) were recorded. The maximal rates of increase and decrease in left ventricular pressure (+dP/dtmax and -dP/dtmax) were determined as parameters of myocardial contractility and relaxation, respectively. Then, the catheter was withdrawn and immediately positioned in the vena cava superior just above the heart, and central venous pressure (CVP) was measured and total peripheral resistance (TPR) was calculated as TPR = MAP - CVP/CO. The abdominal cavity was opened and a flow probe (Transonic, Ithaca, NY, USA) was positioned on the left renal artery [56]. After stabilization, renal blood flow (RBF) was measured. Renal vascular resistance (RVR) was calculated as RVR = (MAP - CVP)/RBF.

Histology

A 1.5 mm thick mid-ventricular slice was fixated in 2% phosphate buffered paraformaldehyde for at least 24 h, dehydrated, and embedded in paraffin for measurement of infarct size and morphometric analysis of cardiac structure. Deparaffinized 5 Im sections were either stained with Sirius Red and fast green to distinguish infarct from viable tissue and to visualize interstitial collagen; with Gomori's silver staining in order to visualize individual myocytes; or with Lectin GSI to visualize capillaries. Methods as well as the measurements of infarct size, myocyte size, capillary density and interstitial fibrosis are described in detail by van Kerckhoven et al. [49]. Infarct size was expressed as percentage of left ventricular circumference.

Kidneys were stained with PASS staining and scored on presence and degree of focal glomerular sclerosis as described before [44].
Aortic sections were stained with Virhoff staining. Aortic hypertrophy was obtained from medial thickness, corrected for lumen diameter, as described before [8].

Cardiomyocyte function

Force measurements were performed in single, mechanically isolated cardiomyocytes as described previously [27, 50]. Briefly, tissue samples were defrosted in relaxing solution, mechanically disrupted and incubated in relaxing solution supplemented with 0.5% Triton X-100 to remove all membrane structures. Single cardiomyocytes were attached with silicone adhesive between a force transducer and a motor: Sarcomere length of isolated cardiomyocytes was adjusted to 2.2 lm. Isometric force was measured in maximally activating calcium solution (pCa = -10- log[Ca2+] = 4.5) and in relaxation solution (pCa 9.0) to determine maximal force generating capacity (Fmax) and passive force (Fpas), and normalized for cardiomyocyte cross-sectional area. On transfer of the cardiomyocyte from relaxing to activating solution, isometric force started to develop. Once a steady state force level was reached, the cell was shortened within 1 ms to 80% of its original length to determine the baseline of the force transducer. The distance between the baseline and the steady force level is the total force (Ftotal). The cell was re-stretched and returned to the relaxing solution, in which a second slack test, of 10 s duration was performed to determine passive force. Maximal force was obtained by subtracting passive force from the total force, i.e. Fmax = Ftotal - Fpas.

Endothelial function

Maximal endothelium-dependent relaxation was measured in vitro in aortic rings obtained from subgroups of the experimental groups, as described before [44]. Briefly, thoracic aorta segments (approximately 2 mm) were cleaned of adherent tissue and mounted in an organ bath with Krebs solution (pH 7.5) containing (in mmol/L): NaCl (120.4), KCl (5.9), CaCl2 (2.5), MgCl2 (1.2), NaH2PO4 (1.2), glucose (11.5), NaHCO3 (25.0), which was kept at 37°C and continuously bubbled with 95% O2 and 5% CO2. After equilibration, viability of smooth muscle cells and endothelium was tested using phenylephrine (PE; 10-6 mol/L). After wash out and another 30 min of stabilization, endothelial function was measured as endothelium-dependent relaxation to cumulative concentration of acetylcholine (ACh; 3 9 10-8 - 3 9 10-4 mol/L) in the vessels precontracted by PE (10-6 mol/L). Area under the curve (AUC) was obtained as measure for endothelial function. Finally, sodium nitroprusside (10-3mol/L) was added to the organ baths to obtain endothelium-independent relaxation. In order to examine the role of cyclo-oxygenase (COX) [44], Ach-induced relaxation was studied in parallel in separate aortic rings in the presence of either 10-6 M indomethacin or 10-5 nimsulin, to inhibit COX1 and COX2, or only COX2, respectively.
Aortic sections were processed for analysis of endothelial nitric oxide synthase (e-NOS)

expression using RT-PCR. For that, total RNA was isolated using TRIzol reagent (Invitrogen Corporation, Breda, The Netherlands) and converted to cDNA by QuantiTect Reverse Transcription (Qiagen, Venlo, The Netherlands). Gene expression was measured with Absolute QPCR SYBR Green ROX Mix (Abgene, Epsom, UK) in the presence of 7.5 ng cDNA and 200 nM forward and reverse primers. qRT-PCR was conducted on the Biorad CFX384 (Biorad, Veenendaal, The Netherlands). Initial denaturation and activation of the DNA polymerase at 95°C for 3 min was followed by 35 cycles with denaturation for 15s at 95°C and annealing and elongation for 30 s at 60°C, followed by a melt curve.
Gene expression levels were corrected for ribosomal protein large (Rplp0). Primers used were endothelial NO synthase (eNOS) forward TCCTAACTTGCCTTGCATCC, eNOS reverse GGCAGCCAAACACCAAAGTC, Rplp0 forward CCTCATACCAGCGACGATTC, Rplp0 reverse ATGTGGAGGAGTCTCACTTC.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Data of rats with infarct < 20% of the left ventricle were excluded from analysis, as these small infarcts are hemodynamically fully compensated [37]. Analyses were performed using SPSS version 16. Parameters were compared using 2-way analysis of variances (ANOVA); effects of cardiac dysfunction as well as renal dysfunction were analyzed first separately, followed by analysis of interaction. This is presented in the figures using different symbols. In case data were not normally distributed, a nonparametric Kruskal–Wallis test followed by a Mann– Whitney U test with correction for multiple comparisons was used. Differences were considered significant at the level of 0.05.
Echocardiography could be obtained in only 3–5 rats per group, and hence results should be regarded as indication, rather than as group evaluation with statistical analysis.

Results

Control rats

Twelve weeks old rats from both strains (MWF = RD; Wistar = control) were sacrificed to obtain baseline values, and general characteristics are presented in Table 1. Food and water intake did not differ between RD rats and controls, nor did urine production. RD rats had a lower body weight than age-matched control. Lung weight and heart weight corrected for body weight were significantly increased in RD. At 12 weeks of age, renal dysfunction was confirmed by increased urinary protein excretion (UPE) and focal glomerulosclerosis (FGS) in RD, but no increase in plasma creatinine levels was observed. RD rats had slightly higher blood pressures, reflected in a 29% increase in left ventricular myocyte cross-sectional area (NS), with normal heart rate and normal left (LVEDP) and right (CVP) cardiac filling pressures. Renal blood flow, however, was strongly reduced in RD due to elevated renal vascular resistance

Table 1. Characterization of control (Wistar) and RD (MWF) rats at 12 weeks of age

	Control	RD
N	5	5
BW (g)	386 ± 7	249 ± 14 [a]
Food intake (g/24h)	15 ± 4	19 ± 1
Water intake (ml/24h)	24 ± 3	26 ± 2
Urine production (g/24h)	15 ± 2	11 ± 1
HW/BW (mg/g)	3.2 ± 0.2	4.5 ± 0.5 [a]
Lung/BW (mg/g)	3.3 ± 0.0	4.8 ± 0.2 [a]
RKW/BW (mg/g)	3.7 ± 0.1	3.7 ± 0.1
UPE (mg/24h)	28 ± 5	76 ± 19 [a]
FGS (AU)	0.4 ± 0.4	1.6 ± 0.4 [a]
Plasma creatinine (mg/l)	31 ± 1	25 ± 4
MAP (mmHg)	89 ± 4	102 ± 3 [a]
LVSP (mmHg)	117 ± 5	131 ± 3 [a]
LVEDP (mmHg)	5.3 ± 2.4	6.2 ± 1.9
HR (beats/min)	359 ± 18	364 ± 14
CVP (mmHg)	1.2 ± 1.9	2.1 ± 1.7
RBF/KW (ml/min/g)	6.0 ± 1.6	2.6 ± 0.6 [a]
Hct (%)	47.6 ± 0.4	44.2 ± 0.3 [a]

HW: heart weight, BW: body weight, RKW: right kidney weight, UPE: urinary protein excretion, FGS: focal glomerulosclerosis, MAP: mean arterial pressure, LVSP: left ventricular systolic pressure, LVEDP: left ventricular enddiastolic pressure, HR: heart rate, CVP: central venous pressure, RBF: renal blood flow, Hct hematocrit ; a: Significant difference between control and RD

(47 ± 12 vs. 17 ± 4 mmHg/ml/min, in RD and control, respectively). Hematocrit was significantly lower in RD rats. Apart from a slightly increased myocyte size, histological analysis showed no signs of adverse left ventricular remodeling in RD; myocyte cross-sectional area 535 ± 71 versus 414 ± 48 lm2; interstitial fibrosis: 5.0 ± 0.9 versus 5.9 ± 0.5%; capillary density: 2,891 ± 337 versus 2,156 ± 141 #/mm2; number of capillaries per cardiomyocyte 1.92 ± 0.28 versus 1.14 ± 0.19, in RD versus control, respectively.

Myocardial infarction

Cardiac dysfunction was induced by experimental myocardial infarction (MI). This procedure resulted in 22% mortality, which occurred mainly within the first 24 h after surgery. Overall

Table 2. General characteristics of the experimental groups at 25 weeks of age

Group	Control	CD	RD	CRD
N	10	6	9	10
BW (g)	482 ± 11	466 ± 14	340 ± 9 [b]	342 ± 8 [b]
Food intake (g/24h)	11 ± 2	13 ± 3	13 ± 1	11 ± 1
Water intake (ml/24h)	21 ± 1	26 ± 5	22 ± 1	22 ± 1
Urine production (g/24h)	15 ± 2	18 ± 3	13 ± 1	14 ± 1
Hct (%)	47.9 ± 0.6	18.7 ± 1.0	45.0 ± 0.8 [b]	45.7 ±0.7 [b]
Plasma [Na] (mmol/l)	137 ± 1	138 ± 1	137 ± 0	136 ± 1
Plasma [K] (mmol/l)	4.4 ± 0.2	4.7 ± 0.2	4.5 ± 0.1	4.7 ± 0.1
Plasma [Cl] (mmol/l)	102 ± 1	103 ± 1	105 ± 1	104 ± 0
Urine [Na} (mmol/l)	92 ± 17	123 ± 36	147 ± 11 [b]	159 ± 22 [b]
Urine [K] (mmol/l)	115 ± 15	125 ± 23	153 ± 9 [b]	152 ± 21 [b]
Urine [Cl] (mmol/l)	101 ± 20	136 ± 40	176 ± 13 [b]	184 ± 25
RKW/BW (mg/g)	3.7 ± 0.1	3.3 ± 0.1 [a]	3.3 ± 0.1 [b]	3.1 ± 0.1 [b]
FGS (AU)	2.4 ± 0.6	3.6 ± 1.2	22.4 ± 2.1 [b]	12.1 ± 1.4 [b]

BW: body weight, Hct: hematocrit, RKW: right kidney weight, FGS: focal glomerulosclerosis, CD: cardiac disease, RD: renal disease, CRD: cardiorenal disease; a: Significant effect of cardiac dysfunction b: Significant effect of renal dysfunction

infarct size was not different in both groups (33 ± 5% in CD and 34 ± 4% in CRD), neither after exclusion of 3 rats with infarcts<20% (34 ± 5% in CD and 38 ± 3% in CRD).
Characteristics of experimental groups at 25 weeks are presented in Table 2. Strain-related characteristics as seen at 12 weeks (Table 1) remained present in sham groups at 25 weeks. Whereas plasma concentrations of electrolytes did not differ between groups, concentrations in urine were significantly higher in rats with RD. MI had no effect on electrolyte concentrations in urine.

Renal effects

Time course of UPE is shown in figure 1. As anticipated, RD rats showed progressive proteinuria with age, which was absent in controls and CD rats. When renal dysfunction was combined with cardiac dysfunction in CRD, besides an initial decrease, proteinuria progressed at the same rate as in RD.
The initial decrease occurred in both MI groups, CD and CRD, at the same percentage. In RD, FGS increased with age from 1.6 ± 0.4 AU at 12 weeks to 22.4 ± 2.1 AU at 25 weeks, and closely matched with the progression of proteinuria, with no additional increase due to

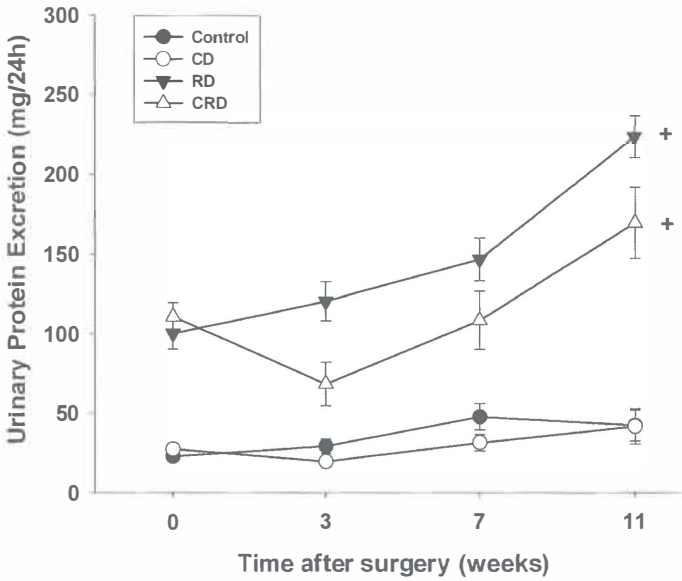

Figure 1. Time course of urinary protein excretion in the experimental groups. Control (n = 10), CD cardiac disease (n = 6), RD renal disease (n = 6), CRD cardiorenal disease (n = 10).
+ Significant effect of renal dysfunction

concomitant cardiac dysfunction. In fact, there was a highly significant, positive correlation between UPE and FGS (r^2 = 0.64, p<0.001). RBF was significantly lower in RD and was not affected by MI in either strain (Table 3). RVR was significantly higher in RD rats than in controls (52.8 ± 13.1 and 13.2 ± 1.9 mmHg/ml/min, respectively), but not significantly affected by MI (CRD 39.5 ± 6.9 mmHg/ml/min; CD 13.5 ± 1.3 mmHg/ml/min). This hemodynamic renal parameter RVR was significantly, negatively correlated with both structural (FGS; r^2 = 0.473, p<0.001) and functional (UPE; r^2 = 0.328, p = 0.002) renal parameters. Creatinine clearance was similar in all groups (control 8.2 ± 0.9; CD 7.3 ± 0.2; RD 10.0 ± 1.3 and CRD 9.1 ± 1.1 ml/min/kg) and decreased approximately 25% with aging irrespective of absence/presence of MI. No significant correlations of creatinine clearance with other renal parameters were found.

Cardiac effects

Effects on cardiac function

Time course of cardiac dysfunction is presented in Figure 2A and B. The EF (Figure 2A) was 85% in sham rats and remained stable over time. At 3 weeks, EF was severely depressed in both groups with MI, and remained at this level for the rest of the study. CO (Figure 2B) showed similar and stable values in sham rats of both strains. Three weeks after MI, CO was strongly depressed and remained low in CRD, but was partly restored in CD. Cardiac function

Table 3. Systemic and cardiac hemodynamics measured at 25 weeks of age

Group	Control	CD	RD	CRD
N	10	6	9	10
HR (beats/min)	331 ± 14	336 ± 35	360 ± 28	416 ± 38
MAP (mmHg)	94 ± 3	88 ± 4	107 ± 4 b	84 ± 5 [a]
LVSP (mmHg)	128 ± 4	123 ± 8	134 ± 3	108 ± 2 [ac]
+dp/dt (mmHg/s)	9091 ± 299	7463 ± 375 [a]	8993 ± 318	8452 ± 455 [a]
-dp/dt (mmHg)	7463 ± 375	6009 ± 432 [a]	6199 ± 513	4377 ± 299 [ac]
RBF/KW (ml/min.g)	7.1 ± 0.8	6.7 ± 0.4	2.8 ± 0.7 b	2.7 ± 0.5 [b]

CD: cardiac disease, RD: renal disease, CRD: cardiorenal disease, HR: heart rate, MAP: mean arterial pressure, LVSP: left ventricular systolic pressure, ±dP/dt: positive/negative first derivative of pressure signal, RBF/KW: renal blood flow/kidney weight; a: Significant effect of cardiac dysfunction; b: Significant effect of renal dysfunction; c: Significant effect of combined cardiorenal dysfunction

and hemodynamics parameters measured 12 weeks after surgery, are presented in Table 3. Heart rate was similar in control rats, rats with CD and rats with RD, but higher ($p = 0.06$) in rats with CRD. RD rats had higher MAP than control, which was significantly reduced after MI. MI reduced left ventricular systolic pressure by 4% in CD, but by 20% in CRD. Whereas MI induced reduction of left ventricular systolic function (+dP/dt), was not exaggerated in CRD compared to CD, diastolic dysfunction, presented as a reduction of relaxation velocity (-dP/dt) as well as increase in tissue Doppler parameter (E/e'), was significantly more pronounced in CRD (Figure 2C). Moreover, heart weight–body weight ratio, as well as lung weight–body weight ratio was significantly higher in the CRD rats (Figure 3). Parameters of congestion, upstream from the injured left ventricle were analyzed and presented in Figure 3. LVEDP was increased by 80% in CD, but almost by 200% in CRD, indicating elevated left ventricular preload. Similarly, left atrial weight increased 3 times more in CRD than in CD. Twice as high right atrial weight only in CRD rats suggests that congestion was not restricted to the damaged left side of the heart. In addition, central venous pressure appeared highest in the CRD rats, although no significant differences could be obtained between the experimental groups.

Effects on myofilament function

Myofilament function of isolated skinned fibers from the viable left ventricular free wall is presented in Figure 4. Whereas active force was similar in all groups, passive force increased after MI. This increase was found statistically significant in CRD, but not in CD.

Effects on cardiac structure

Histological analysis of left ventricle of 25 weeks old sham rats revealed similar effects to

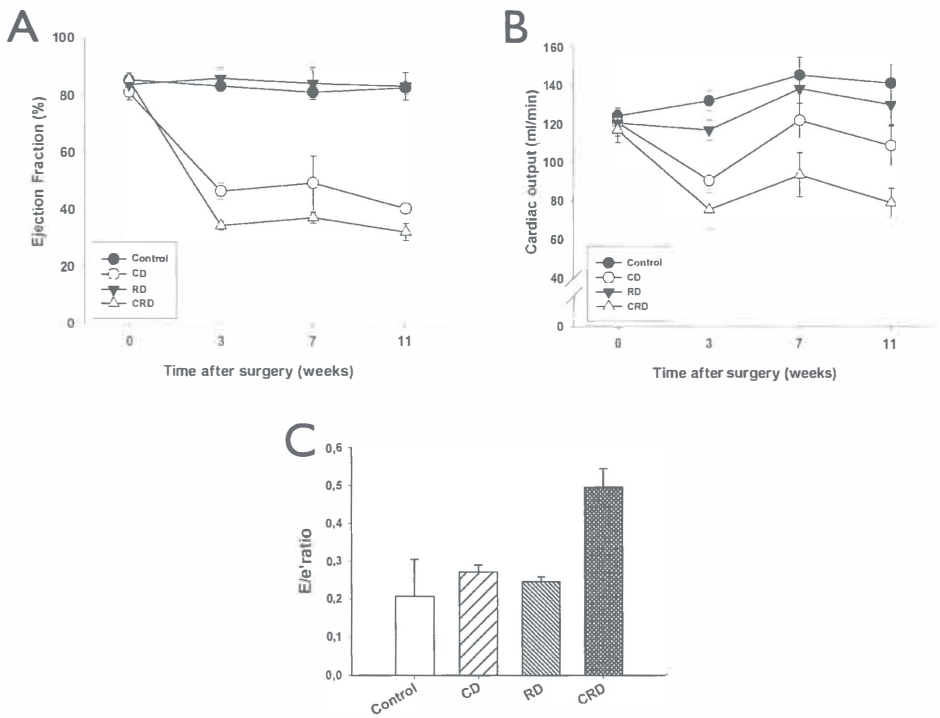

Figure 2. *Cardiac function parameters obtained by echocardiography. E/e' early filling velocity (E) to mitral valve septal annular velocity (e') ratio. Control (n = 5), CD: cardiac disease (n = 3), RD: renal disease (n = 5), CRD: cardiorenal disease (n = 4)*

those of 12 weeks old rats (Figure 5). MI significantly reduced capillary density to the same extent in CD and CRD. Similarly, interstitial collagen and myocyte size were increased due to MI, but without differences between CRD and CD. Furthermore, no differences between the groups are found for number of capillaries per myocyte (range 1.8–2.3). Myocyte size in the right ventricle was significantly and exponentially ($p<0.001$) increased in rats with CRD (Figure 5), and was significantly and positively correlated with congestion parameters, such as LVEDP, atrial weight and lung weight. Hence, right ventricular myocyte size may better explain the increased heart weight body weight ratio ($r^2 = 0.418$; $p<0.001$) in rats with CRD than left ventricular myocyte size ($r^2 = 0.002$; $p = 0.797$).

Circulatory effects

Effects on blood

Before MI, plasma renin activity (PRA) was significantly lower in RD compared to control rats; 4.4 ± 0.5 versus 8.0 ± 0.8 ngAngI/ml/h. MI induced a significant increase in PRA only in CRD rats (altered PRA from before- to 7 weeks post-MI: CRD +6.2 ± 1.6; CD -2.1 ± 2.5; RD -1.2

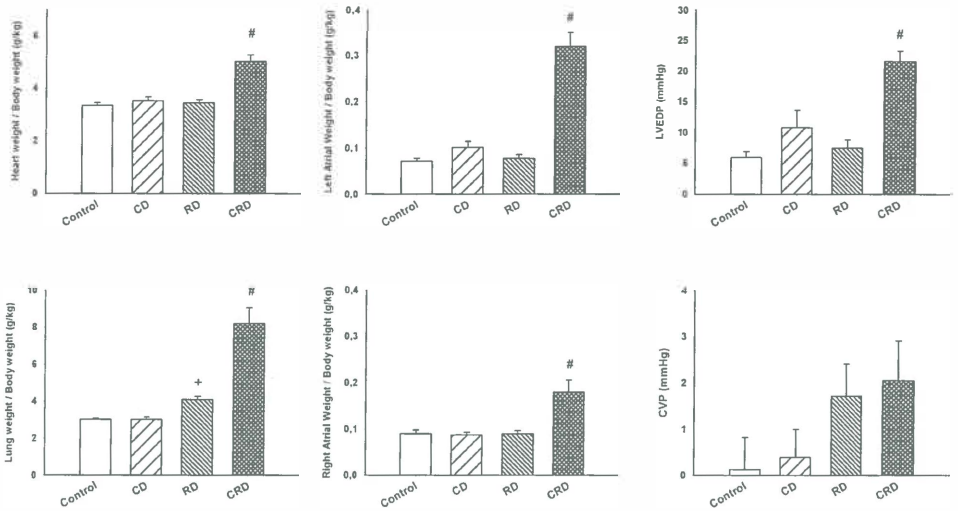

Figure 3. Parameters of congestive heart failure. Control (n = 10), CD: cardiac disease (n = 6), RD: renal disease (n = 9), CRD: cardiorenal disease (n = 10). LVEDP: left ventricular enddiastolic pressure, CVP: central venous pressure. + Significant effect of renal dysfunction; # Significant effect of combined cardiorenal dysfunction

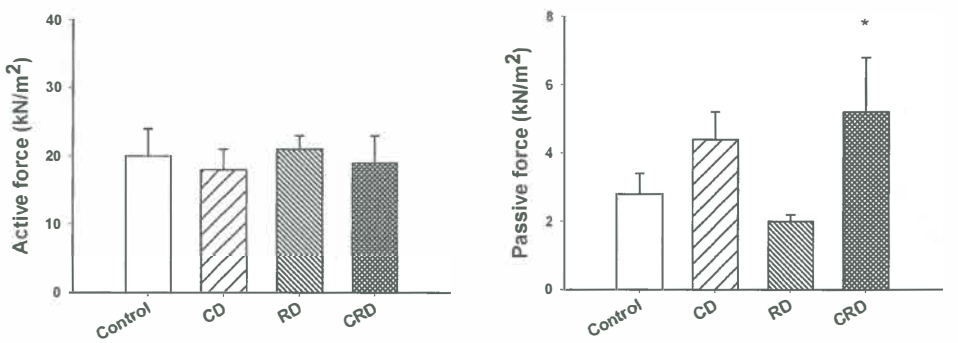

*Figure 4. Active and passive force development in isolated myofibrils. Control (n = 5), CD: cardiac disease (n = 5), RD: renal disease (n = 5), CRD: cardiorenal disease (n = 5). *Significant effect of cardiac dysfunction*

± 1.2; control -2.5 ± 1.0 ngAng I/ml/h). This increased PRA was significantly correlated with LVEDP (r^2 = 0.461, p<0.001), lung weight (r^2 = 0.515, p<0.001) and right ventricular myocyte size (r^2 = 0.175, p = 0.042), but not with renal parameters, such as proteinuria, creatinine and renal blood flow. Before MI, there was no difference in BNP levels between groups. MI induced highest increase of BNP in CRD rats (altered BNP from before to 7 weeks post-MI: CRD 1.1 ± 0.2; RD 0.3 ± 0.4; CD 0.5 ± 0.2; control 0.6 ± 0.2 ng/ml). BNP values 7 weeks post-MI were positively correlated with lung (r^2 = 0.184, p = 0.014) and atrial weights (r^2 = 0.215, p=0.008

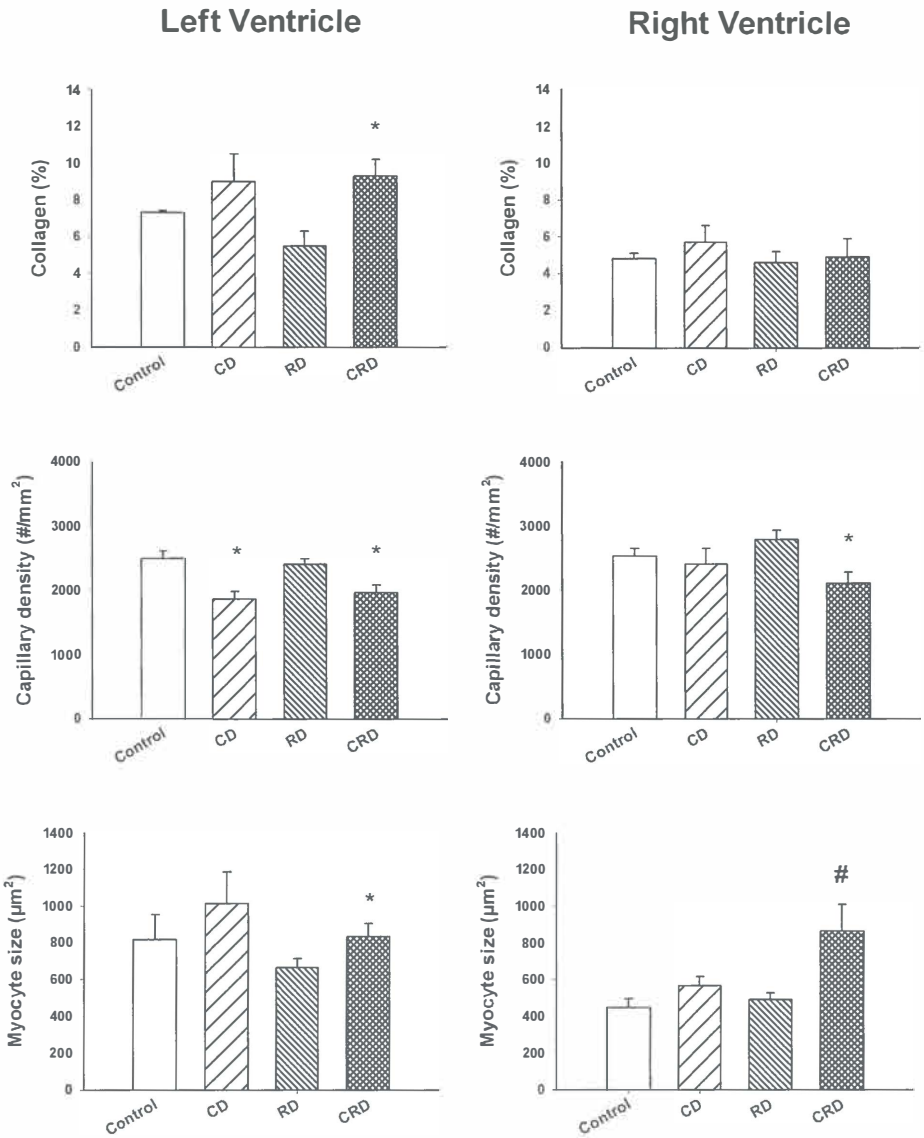

*Figure 5. Left and right ventricular remodeling presented as interstitial collagen, capillary density and myocyte hypertrophy. Control (n = 10), CD: cardiac disease (n = 5), RD: renal disease (n = 9), CRD: cardiorenal disease (n = 10). *Significant effect of cardiac dysfunction; #Significant effect of combined cardiorenal dysfunction*

and $r^2 = 0.138$, $p = 0.040$ for left- and right atrium, respectively), but not with renal parameters. MI in CD did not affect hematocrit, nor number of red blood cells (Table 4). However, number of white blood cells was significantly reduced after MI. In RD, lower hematocrit levels shown at 12 weeks of age, were also observed at 25 weeks, and matched with lower numbers of red

blood cells (Table 4). Number of white blood cells was pronouncedly reduced in RD. In CRD, MI did not affect hematocrit, number of red blood cells, or number of white blood cells. Platelet numbers were similar in all groups.

Vascular effects

There were no differences between groups with regard to aortic media/lumen ratio at 12 weeks after MI (control 0.23 ± 0.03; CD 0.23 ± 0.04; RD 0.25 ± 0.2: CRD 0.28 ± 0.02). There was a slight increase in total peripheral resistance in CD rats from 0.71 ± 0.07 to 0.95 ± 0.10 mmHg min/ml, and in CRD from 0.81 ± 0.07 to 1.04 ± 0.18 mmHg min/ml. A significant correlation was found between TPR and left ventricular congestion parameters, LVEDP and left atrial weight ($r^2 = 0.328$, $p = 0.026$; $r^2 = 0.355$, $p = 0.016$, resp.), but not with pulmonary and right ventricular congestion parameters, nor with renal parameters. In aortic rings, endothelium-independent relaxation, obtained by response to sodium nitroprusside, was reduced by 23% (NS) in CD and RD and by 53% in CRD rats ($p<0.05$). Moreover, endothelium-independent relaxation was significantly correlated with congestion parameters LVEDP, left atrial weight and lung weight, but not with any of the renal parameters. Endothelium-dependent relaxation, obtained by the response to acetylcholine, is presented in figure 6A. RD caused a significant reduction in endothelium-dependent relaxation (AUC control 99.0 ± 14.8 AU; RD: 54.6 ± 11.9 AU), which was maintained after correction for endothelium-independent relaxation. CD did not significantly impair endothelium-dependent relaxation (AUC 93.0 ± 37.8 AU). Although not statistically significant, an additional effect might be seen in the vessels from CRD rats (AUC 32.8 ± 9.4 AU). Interestingly, endothelium-dependent relaxation was significantly correlated with both renal dysfunction parameters as well as left ventricular overload parameters (figure 7), but not with systemic hemodynamic parameters, such as TPR, MAP and CVP. Endothelial NO-synthase expression was slightly increased in aortas of rats with renal dysfunction, but without additional effects of MI (eNOS/Rplp0 in control 0.82 ± 0.15; CD 0.75 ± 0.27; RD 1.11 ± 0.20; CRD 1.18 ± 0.17).

Effects of COX inhibition on Ach-induced relaxation is presented in figure 6B. In control as well as RD, COX2 inhibition by nimsulide enhanced the Ach-induced relaxation, whereas COX1 and COX2 inhibition by indomethacin had no effect, indicating a COX1 mediated vasodilation compensated by COX2 mediated vasoconstriction. MI had similar effects in CD as well as CRD; no effect of nimsulide, but increased vasorelaxation on indomethacin.

Discussion

Even mild renal dysfunction substantially increases the risk of cardiovascular morbidity or mortality, and many patients with heart failure suffer from concomitant renal dysfunction, supporting a strong interaction between heart and kidneys. Primary chronic kidney disease

Table 4. Effects on hematocrit (Hct), number of red- (RBC) and white (WBC) blood cells, and platelets

Group	Control	CD	RD	CRD
N	10	6	9	10
Hct (%)	47.8 ± 0.6	48.7 ± 1.0	45.0 ± 0.8 [b]	45.7 ± 0.7 [b]
RBC (#.1012/l)	9.4 ± 0.1	9.4 ± 0.1	7.9 ± 0.2 [b]	7.8 ± 0.1 [b]
WBC (#.1012/l)	10.7 ± 0.8	8.7 ± 0.6 [a]	2.2 ± 0.2 [b]	2.3 ± 0.1 [b]
Platelets (#.1012/l)	739 ± 68	852 ± 49	887 ± 44	832 ± 40

CD: cardiac disease, RD: renal disease, CRD: cardiorenal disease, # number; a: Significant effect of cardiac dysfunction; b: Significant effect of renal dysfunction

is associated with extremely high cardiovascular risk [36]. The interaction between heart and kidneys in this relatively high-risk group of patients was studied in an animal model mimicking the clinical condition; rats that spontaneously develop progressive renal dysfunction, subjected to experimentally induced heart failure. The main findings were: (1) cardiac, but not renal dysfunction was exaggerated in combined cardiorenal dysfunction; (2) cardiac dysfunction was characterized by systolic and diastolic dysfunction with cardiopulmonary congestion and right ventricle hypertrophy; (3) adverse left ventricular remodeling and myofilament dysfunction could not explain the deteriorated cardiac function in cardiorenal disease; (4) vascular endothelial dysfunction may provide a link between renal and cardiac dysfunction.

Rat model for cardiorenal disease

As anticipated from literature [35], renal dysfunction in 12 weeks old RD rats was confirmed by functional, structural and hemodynamic changes, represented by increased UPE and FGS and reduced renal blood flow, respectively. Furthermore, hematocrit levels were reduced, which is in agreement with previous findings in rats [1], as well as in patients with chronic renal disease [23, 46], and notified as a "novel" risk factor in cardiorenal disease [47]. RD coincided with mildly increased MAP and normal CVP [24]. Combining this model with experimental myocardial infarction, a well-established model of cardiac dysfunction [37, 38], may therefore provide a suitable animal model to study chronic renal dysfunction as predisposition for high cardiovascular risk.

Renal dysfunction in cardiorenal disease

There was no effect of cardiac dysfunction on progression of renal dysfunction as evidenced by functional (UPE), hemodynamic (RVR) and structural (FGS) parameters. Apart from the initial decrease, the progression of UPE in CRD was similar to that in RD. Since this initial decrease occurred to the same extent in CD and CRD rats, it might be attributed to reduction in MAP shortly after MI. The absence of progressive proteinuria is in contrast to results reported in rats after unilateral nephrectomy and MI [48], but in accordance with the commonly used

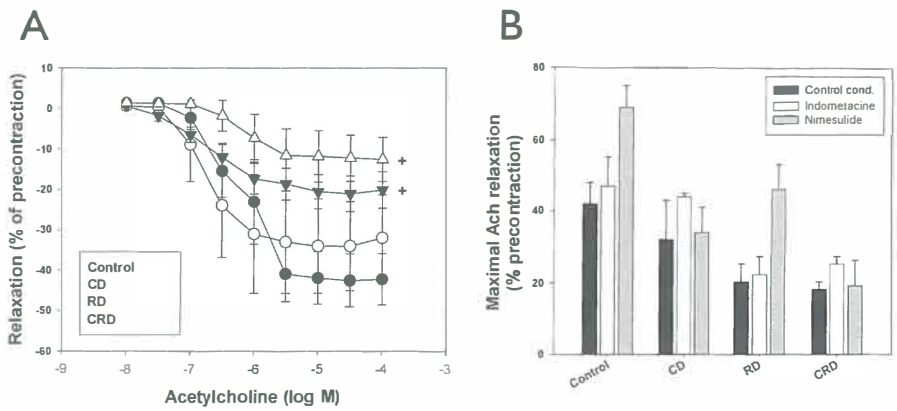

Figure 6. Parameters of endothelial function. A Concentration-dependent relaxation to acetylcholine in phenyleprine-precontracted aortic rings. B Effect of COX inhibition on maximal Achinduced relaxation. Control (n = 6), CD: cardiac disease (n = 4), RD: renal disease (n = 6), CRD: cardiorenal disease (n = 5). INDO indomethacin, NIM nimesulide; +Significant effect of renal dysfunction

cardiorenal model of rats with subtotal nephrectomy and MI [55, 56]. Renal dysfunction in RD and CRD resulted in increased FGS in comparison to control and CD; however, it was lower in CRD than in CD. When taking the strong correlation between UPE and FGS, it seems that this difference may have also occurred in the early post-MI phase. RVR was severely increased in RD compared to control, but without additional increase in CRD. All these functional, hemodynamic and structural parameters of renal dysfunction were significantly inter-correlated, supporting mutual interaction of renal parameters and substantiating the observation of absence of progressive renal dysfunction due to concomitant cardiac dysfunction. In contrast, creatinine clearance, as an estimate of glomerular filtration rate, was reduced over time, but not different between experimental groups. This would support recent suggestions that proteinuria and reduced glomerular filtration rate may represent different types of renal dysfunction, rather than being a measure of increased severity [5]. Finally, no correlations were found between renal parameters and cardiac parameters, indicating absence of a direct relationship in the present study. Although not accelerated by concomitant cardiac dysfunction, renal function still was declining over time in CRD. It is generally acknowledged that worsening of renal function can lead to worsening of cardiac function and outcome in patients, as reviewed by Damman and coworkers [6]. However, to our knowledge there are no clinical studies available investigating the opposite relation: comparing worsening of renal function in patients with or without concomitant cardiac dysfunction.

Cardiac dysfunction in cardiorenal disease

Calculated CO provided a first indication of more severe cardiac dysfunction in rats with primary renal dysfunction. Accelerated cardiac dysfunction in CRD, as indicated by lower CO, was substantiated by exponential increases in left ventricular overload parameters, congestion

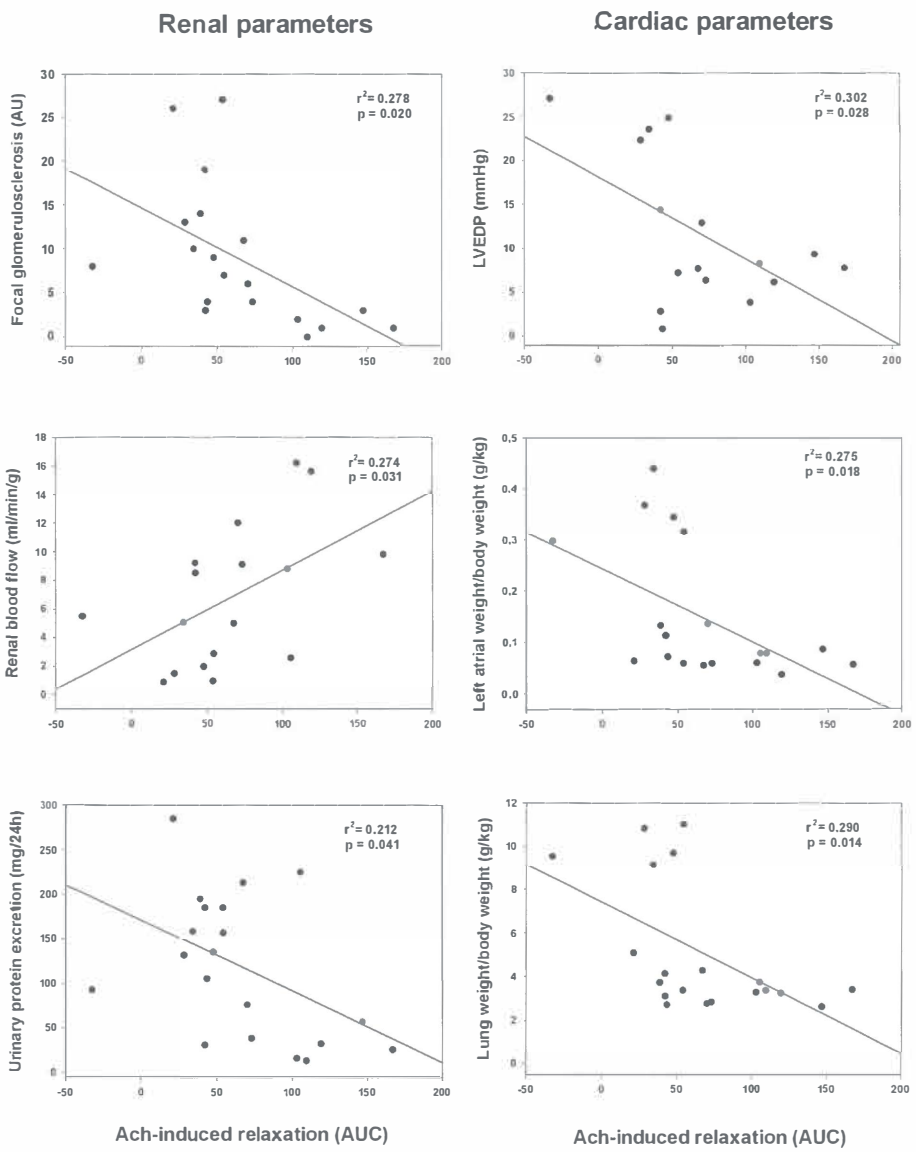

Figure 7. Statistically significant correlations between endothelial function and renal as well as cardiac parameters

and failure. Comparing our results to available literature reveals that accelerated cardiac dysfunction was not observed in unilateral nephrectomy + MI [48] or in 5/6 nephrectomy + MI [56], whereas rats with temporal L-NNA infusion, in addition to 5/6 nephrectomy, did show signs of more severe cardiac dysfunction [1]. However, one recent study is showing aggravation of cardiac remodeling with concomitant renal dysfunction [10]. Unfortunately, this

study did not include rats with only 5/6 nephrectomy or sham MI rats. Moreover, control rats did not show cardiac dysfunction after MI as measured by EF or LVEDD, and changes in cardiac function and remodeling could well be attributed to the larger infarct sizes in the cardiorenal group [10]. Since the congestion in systolic heart failure is related to renal dysfunction and increased mortality [7], many studies focused on systolic dysfunction. However, in a substantial proportion of cardiorenal disease patients, cardiac dysfunction is characterized by diastolic rather than systolic dysfunction; heart failure with preserved ejection fraction [29, 32]. Data from the present study indicate that rats with CRD may display diastolic heart failure indicated by stronger reduction in cardiac output at similar ejection fraction, increased E/e' ratio and signs of congestion. This was accompanied by right ventricular overload, indicative for pulmonary hypertension, as commonly observed in cardiorenal diseased patients [26]. In a recent study, it was argued that diastolic dysfunction only develops into congestive diastolic heart failure because of underlying renal insufficiency [51], which underscores the relevance of our model.

Underlying mechanisms

Adverse cardiac remodeling

In 12 weeks old MWF, a cardiac phenotype was indicated from higher heart weight–body weight ratios associated with increased left ventricular systolic pressure at normal enddiastolic pressure. Three months of CRD resulted in pronouncedly increased cardiac weight, suggesting adverse cardiac remodeling [43]. However, besides a minor, and possibly blood pressure-related left ventricular myocyte hypertrophy, adverse remodeling of the left ventricle was not observed either at 12 or at 25 weeks. Thus, adverse left ventricular remodeling neither was a predisposition to develop accelerated cardiac dysfunction in CRD, nor could be associated with the exaggerated cardiac dysfunction in CRD, once established. This is in accordance with results in rats with 5/6 nephrectomy + MI [34, 56], but in contrast to a recent study of Dikow et al. [10]. In this latter study, however, effects on remodeling were only observed in the border zone of the scar and not in the remote areas, and hence would match with our results obtained in remote areas. In the present study, increased pulmonary weight suggested pulmonary congestion and right ventricular overload. This was evidenced by a significant increase in right ventricular myocyte size, indicating right ventricular concentric hypertrophy, and could be associated with the activated renin angiotensin system in CRD. A higher correlation between total ventricular weight and right-, compared to left ventricular myocyte size, supports an important contribution of the right ventricle to increased heart weight in CRD. Moreover, right ventricle overload might have also contributed to the dysfunction of LV [4].

Myofilament function

Cardiac diastolic dysfunction could be caused by various mechanisms [11], including altered function of contractile proteins within the cardiomyocyte. Whereas active force was similar in all groups, passive force was significantly increased in CRD. Correlation with impaired left

ventricular relaxation indicated intrinsic diastolic dysfunction rather than fibrosis related stiffness of the left ventricle. High cardiomyocyte stiffiness has been reported in diastolic dysfunction patients and correlated significantly with LVEDP [2].

Anemia

Irrespective of concomitant cardiac dysfunction, MWF showed anemia, indicated by lower hematocrit as well as number of red blood cells. Since white blood cell number is even more pronouncedly decreased, anemia may result from general bone marrow dysfunction rather than from hemodilution or erythrocyte-specific mechanisms, such as erythropoietin-resistance [23]. This is supported by results of a pilot study of culturing bone marrow-derived endothelium progenitor cells [33], showing less than 10% yield in MWF compared to Wistar. All aspects, including anemia [23, 25], erythropoietin-resistance [39], reduced number/and or function of endothelium progenitor cells [3, 16, 40], general bone marrow dysfunction [21, 22], are commonly described in renal dysfunction. However, as no additional effects were observed in CRD rats compared to CD and RD rats, this does not provide an explanation for the accelerated cardiac dysfunction in primary renal dysfunction.

Vascular function

Endothelial dependent relaxation measured in aortic rings served as a biomarker for endothelial dysfunction in more distant vascular beds. MI has been associated with reduced endothelium-dependent relaxation in aortic rings [13, 53]. Moreover, impaired endothelium-dependent relaxation in aortic rings in MWF [44] could be associated with endothelial dysfunction in coronary, but not in mesenteric arteries [15]. There is growing evidence of the role of endothelium in regulation of cardiac diastolic function [54]. It is believed that low availability of NO produced by endothelial NO synthase leads to decrease in diastolic function, cardiac hypertrophy and fibrosis, whereas enhancement of this enzyme attenuates these changes. Endothelium derived NO has been shown to have positive effects not only on diastolic, but also on systolic function [18]. Moreover, the endothelial dysfunction might limit the cardioprotective effect of erythropoietin [42]. Therefore, exaggerated endothelial dysfunction in CRD might have contributed to further impairment of cardiac dysfunction in these rats. However, as eNOS expression in the present study was similar in RD and CRD and even slightly higher compared to control and CD, impaired eNOS would not explain the endothelial dysfunction in CRD. Similarly, MI induced altered COX-activity was comparable in CD and CRD and hence may not have contributed to endothelial dysfunction in CRD. Unfortunately, to our knowledge no data are available about endothelial function in renal arteries in MWF. Interestingly, aortic endothelial function was the only parameter that significantly correlated with renal as well as cardiac parameters, suggesting a mediating role for the vasculature. This suggested role may be supported by the finding that endothelial function predicts individual susceptibility to renal damage in 5/6 nephrectomy [14, 30, 31].

Study limitations

Although the present study may throw new light on the interaction between heart and kidneys in cardiorenal disease, it may have some limitations as well: the MWF rat strain used in this study is an inbred model based on proteinuria, which could have caused other unknown genotypical/ phenotypical changes as well; we cannot fully exclude primary pulmonary disease in this strain. Moreover, as most inbred Wistar-origin models, MWF rats had substantially lower body weights at the same age than outbred Wistar. Although a clear explanation is to our knowledge not known, it may trouble either/not correcting for body weight. Furthermore, although proteinuria, focal glomerulosclerosis, mild hypertension and reduced renal blood flow are well known in the MWF's, reduced GFR as often seen in cardiorenal patients was not present in the MWF. Finally, the first indication for exaggerated cardiac dysfunction came from echocardiographical measurements only performed in a subgroup of rats, which clearly hampers proper statistical analysis.

Conclusion

In conclusion, experimental MI did not exaggerate renal dysfunction in the proteinuric MWF rat. However, cardiac dysfunction was exaggerated by this primary renal dysfunction. The accelerated cardiac dysfunction could be characterized by elevated left ventricular preload, as well as pulmonary congestion, right ventricular overload and right ventricular hypertrophy. This animal model may provide an important step to understand the complex clinical condition of cardiorenal disease. Finally the study provides evidence that vascular endothelial dysfunction may play a mediating role in this cardiorenal interaction.

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Chapter 5

Use of gated ^{13}N-NH_3 micro-PET to examine left ventricular function in rats

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Abstract

Introduction. Myocardial perfusion gating techniques offer possibility of measurement of left ventricular end systolic (ESV) and end diastolic volume (EDV) and left ventricular ejection fraction (LVEF) in clinical and pre-clinical trials. The aim of this study was to evaluate left ventricular volumes (LVV) and LVEF with ^{13}N-NH_3 in comparison with the reference ^{18}F-FDG in different rat models.

Methods: In this study 18 male Wistar rats, 12 control rats and 6 rats with myocardial infarction (MI) were imaged with micro-PET. The rats were scanned with gated ^{13}N-NH_3 and ^{18}F-FDG sequentially for the assessment of LVV and LVEF. A validated 3-dimensional segmentation algorithm was used to calculate LVV and LVEF.

Results: Mean LVEF measured with ^{13}N-NH_3 was 45.6±8.9 and 75.3±9.4%, mean ESV was 0.40±0.12 and 0.14±0.11 ml and mean EDV was 0.53±16 and 0.75±0.18 ml for MI and control rats respectively. Moderate to good correlations were observed between values of ^{13}N-NH_3 and ^{18}F-FDG for calculation of ESV ($r = 0.80$, $P<0.0001$, SEE = 0.10), EDV ($r = 0.63$, $P=0.005$, SEE = 0.14) and LVEF ($r =0.84$, $P<0.0001$, SEE = 9.5). LVEF measured with ^{13}N-NH_3 was significantly lower in MI rats in comparison to measurement with ^{18}F-FDG (45.6 ± 8.9 vs 54.9 ± 9.3 %; $p = 0.04$).

Conclusion: Correlations were moderate to good for the assessment of ESV, EDV and LVEF between gated ^{13}N-NH_3 and ^{18}F-FDG. LVEF was underestimated with gated ^{13}N-NH_3 in rats with myocardial infarction. In healthy rats LV volumes and LVEF can be measured reproducible with both approaches.

Introduction

Animal models are widely used in studies on heart disease and novel treatment therapies. However, the possibilities of non-invasive assessment of cardiac function in small animals is still limited. Positron emission tomography (PET) has been proven to be an useful and accurate tool for detection of stunning, ischemia and for measuring perfusion [1]. In addition, PET can be used to visualize cardiac perfusion and metabolism and the calculation of left ventricular volumes (LVV) and ejection fraction (LVEF) by the application of cardiac gating techniques and specific PET tracers [2,3]. A combined measurement of different parameters is a powerful way to monitor cardiac function and perfusion in a non-invasive way. This may be helpful to evaluate the effectiveness of various treatment strategies in small animal models of cardiac dysfunction. Previously, these measurements had to be made sequentially with use of different tracers, characterized by their own specific properties. PET measurements performed with ^{13}N-NH_3 are currently predominantly used as an accurate non-invasive diagnostic technique for quantification of myocardial perfusion in the clinical setting [4]. Simultaneous measurement of myocardial perfusion, LVV and LVEF with ^{13}N-NH_3 using a combination of dynamic and ECG-gated acquisition protocols has been reported earlier in human studies, but not in animal studies [5,6]. Even though clinical results are very promising, cardiac nuclear imaging in small animals remains challenging due to the small size of the animal and consequent small heart volume.

^{18}F-FDG has proven itself to be a reliable tracer for the assessment of LVV and LVEF and myocardial viability, in rats as well as in humans [1,2,5,7] and is therefore the preferred method for these measurements. However a gated ^{13}N-NH_3 PET measurement in small animals has not been evaluated so far. The aim of this study was to evaluate gated ^{13}N-NH_3 for the assessment and reproducibility of LVV and LVEF measurements in rats in comparison with the reference method gated ^{18}F-FDG PET using micro-PET.

Materials and methods

Animals

The study was performed in 18 male Wistar rats (Harlan, The Netherlands). Animals were housed in groups under standard conditions at 12h light/dark cycle until they reached 12 weeks of age. All animals received standard diet and water ad libitum during the study. At 12 weeks of age rats were subjected to myocardial infarction (MI) (n = 6) by coronary artery ligation as described before [8]. Twelve controls were included. Approximately 11 weeks after MI surgery, the rats were subjected to micro-PET scanning (PET) to study LV volumes and LVEF. The rats were scanned using the radiotracers ^{13}N-NH_3 and ^{18}F-FDG sequentially within a single anaesthetic period. Within two weeks PET studies were repeated in 9 control rats to

study reproducibility of the measurements.
Myocardial infarction size was evaluated in the paraffin sections stained with Sirius Red/ Fast Green and expressed as the mean of the inner and outer percentage of scar tissue to the inner and outer circumference of the left ventricle. All animal experiments were performed by licensed investigators in compliance with the Law on Animal Experiments of The Netherlands. The protocol was approved by the Committee on Animal Ethics of the University of Groningen.

Data Acquisition

All rats were scanned while under 1.5% isoflurane anaesthesia and maintained in a fixed supine position. A heating pad was used to maintain a constant body temperature. PET tracers were intravenously injected into the penile vein. ^{13}N-NH_3 was injected first, followed by ^{18}F-FDG with a time interval of 45 minutes. The radiotracers were administered as a 0.4 - 0.6 mL bolus, with an average dose of 48.8 ± 13.8 MBq for ^{13}N-NH_3 and 51.0 ± 13.0 MBq for ^{18}F-FDG. The camera (micro-PET Focus 220; Siemens/Concorde) was started simultaneously with injection of ^{13}N-NH_3. To enable the subtraction of residual background activity from the ^{13}N-NH_3 study, the ^{18}F-FDG acquisition protocol was started 5 minutes before the actual ^{18}F-FDG injection. The interval between the start of the ^{13}N-NH_3 and ^{18}F-FDG acquisition was 1 hour. A separate transmission scan was acquired for attenuation correction. Different list-mode protocols were used. A 20 minutes list-mode acquisition protocol was used for ^{13}N-NH_3 imaging and a 95 minutes list-mode acquisition protocol was used for ^{18}F-FDG imaging. During the whole protocol cardiac excitation was recorded and stored using the BioVet system. Three ECG electrode leads were placed on both for-legs and left hind-leg.

Data Processing

PET data was reframed into a gated sequence of 8 bins. The data were reconstructed per bin using an iterative reconstruction algorithm (4 iterations, 16 subsets, 2-dimensional ordered-subsets expectation maximization). Datasets were fully corrected for random coincidences and scatter. Images were processed using software programmed in MATLAB and in C programming language to obtain end-systolic volume (ESV), end-diastolic volume (EDV) and LVEF values. First re-angulation of the original PET images was performed resulting in typical vertical- and horizontal long axis and short axis views. A validated 3-dimensional segmentation algorithm was then used to identify the endocardial contours to calculate LV volumes [1]. Examples of re-angulated ^{13}N-NH_3 and ^{18}F-FDG images obtained from a control and a MI animal are shown in figure 1.

Statistical analysis

Analyses were performed using standard statistical software (GraphPad Prism, Excel). ESV, EDV and LVEF values as measured with micro-PET are shown as mean ± SD. A paired two-sided

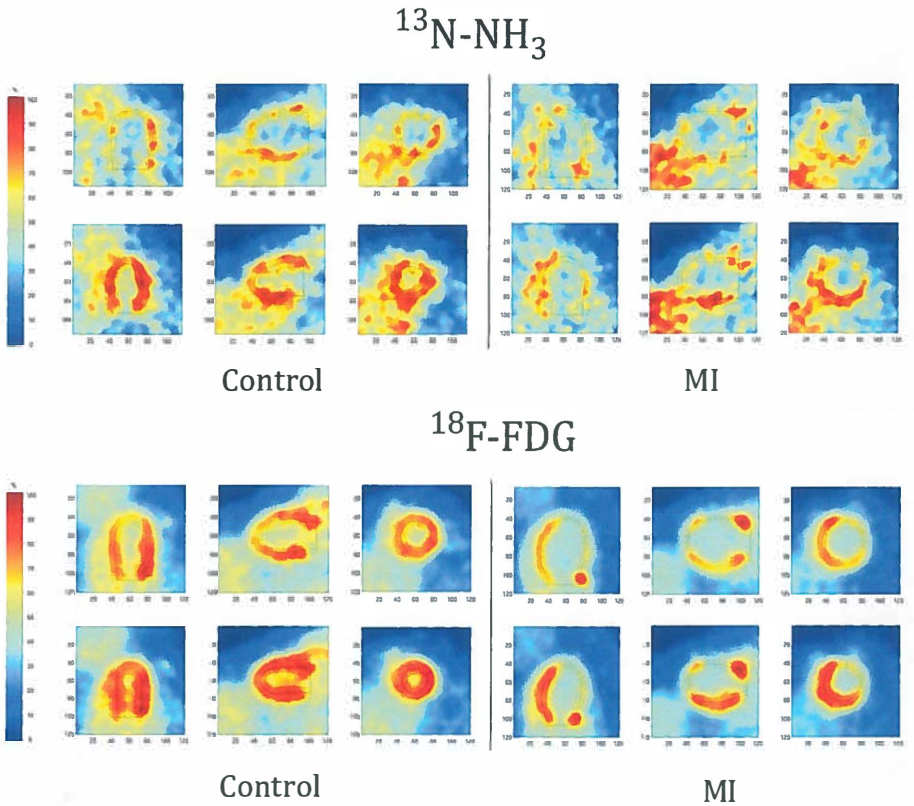

Figure 1. *Examples of re-angulated heart images from a control and an MI animal obtained with gated ^{13}N-NH_3 and ^{18}F-FDG*

Student t test was used to compare results obtained from the two radiotracers. Correlations between ^{18}F-FDG and ^{13}N-NH_3 were performed using Pearson correlation coefficients. Agreement between ^{18}F-FDG and ^{13}N-NH_3 values was evaluated using Bland-Altman analyses [9].

The validity of ^{13}N-NH_3 LV function measurements was analysed by determining precision, accuracy and bias. The limits of agreement were set at ± 2 SD.

Results

There were no significant differences in body weight (364 ± 94 vs. 423 ± 72 g for control and MI rats respectively) or heart rates (354 ± 22 vs. 349 ± 49 beats per minute for control and MI rats respectively) during PET imaging between groups. Mean MI size in MI group was 37 ± 12%. Good reproducibility was observed for ESV, EDV and LVEF when control rats were scanned on different days for gated ^{13}N-NH_3 as well as ^{18}F-FDG PET (table 1).

Table 1. *Reproducibility of LV volumes and LVEF measurements with gated ^{13}N-NH_3 and ^{18}F-FDG micro-PET*

	test	re-test	SAD	P	n
^{13}N-NH_3					
ESV (mL)	0.12 ± 0.05	0.11 ± 0.03	0.21	0.94	6
EDV (mL)	0.47 ± 0.07	0.49 ± 0.12	0.59	0.32	6
LVEF (%)	75.4 ± 7.8	78.8 ± 6.0	41	0.29	6
^{18}F-FDG					
ESV (mL)	0.07 ± 0.02	0.08 ± 0.03	0.12	0.32	5
EDV (mL)	0.41 ± 0.09	0.39 ± 0.22	0.48	0.74	5
LVEF (%)	81.5 ± 3.5	75.1 ± 5.1	45	0.24	5

ESV: end-systolic volume; EDV: end-diastolic volume; LVEF: left ventricular ejection fraction; SAD: sum of absolute differences.

The mean values of LVV and LVEF are presented in table 2. There were no significant differences observed between values obtained with gated ^{13}N-NH_3 and ^{18}F-FDG PET in the control group, or in the group as a whole for ESV, EDV or LVEF. Both the control and the MI group showed a lower LVEF when compared to ^{18}F-FDG. This difference was significant in the MI group ($P = 0.04$). EDV and ESV values were higher, but not significantly, in both groups when compared with ^{18}F-FDG measurements. When comparing the values obtained with different tracers, significant correlations were observed between values of gated ^{13}N-NH_3 and ^{18}F-FDG PET for ESV ($r = 0.80$, SEE = 0.10, $P < 0.0001$), EDV ($r = 0.63$, SEE = 0.14 , $P = 0.0048$) and LVEF ($r = 0.84$, SEE = 9.5, $P < 0.0001$) (figure 2).

The agreement analysis between measurements is presented in figure 3. Small mean differences of 0.04 ml for and 3% for LVEF (95% confidence intervals -0.31 - 0.23 ml for LVV and -15 - 21 % for LVEF) were found (table 3). Both mean values of ESV and EDV were higher with when measured with ^{13}N-NH_3 in comparison to ^{18}F-FDG PET, whereas mean LVEF was lower when obtained from ^{13}N-NH_3 images. When determining the strength of the relation we found that it was moderate to good for EDV ($r = 0.63$), ESV ($r = 0.80$) and LVEF ($r = 0.84$). Agreement proportions of 67 % were achieved within 0.1 ml for ESV, 83% within 0.2 ml for EDV and 72% within 10% for LVEF.

Discussion

In this study we showed that LV function measurements (ESV, EDV and LVEF) can be obtained with gated ^{13}N-NH_3 micro-PET images in rats, both healthy and rats with an induced myocardial infarction. In the total group of rats a moderate agreement for ESV and EDV and good

Table 2. Overview of LV volumes and LVEF obtained with gated ^{13}N-NH_3 and ^{18}F-FDG micro-PET.

Parameter	^{13}N-NH_3	^{18}F-FDG	P	n
ESV (mL)				
Control	0.14± 0.11	0.12 ± 0.07	0.45	12
MI	0.40 ± 0.12	0.32 ± 0.14	0.17	6
Total	0.23 ± 0.17	0.19 ± 0.14	0.12	18
EDV (mL)				
Control	0.53 ± 0.16	0.49 ± 0.16	0.38	12
MI	0.75 ± 0.18	0.70 ± 0.24	0.58	6
Total	0.60 ± 0.19	0.56 ± 0.21	0.28	18
LVEF (%)				
Control	75.3 ± 9.4	74.9 ± 10.2	0.88	12
MI	45.6 ± 8.9	54.9 ± 9.3	0.04	6
Total	65.4 ± 17.0	68.3 ± 13.7	0.20	18

ESV: end-systolic volume; EDV: end-diastolic volume; LVEF: left ventricular ejection fraction

agreement for LVEF was observed when comparing the two gated-based imaging methods. Both ESV and EDV obtained from ^{13}N-NH_3 images were slightly higher, but not significant as compared to ^{18}F-FDG-derived values. There was an underestimation of LVEF with ^{13}N-NH_3 when compared to ^{18}F-FDG observed, which was significant, in the MI group.

Direct comparison of gated ^{18}F-FDG with ^{13}N-NH_3 has not been studied before in a small animal model. The effect of the algorithm used on ^{13}N-NH_3 derived images to calculate EDV, ESV and LVEF and the differences in properties of the tracers itself may result in small differences of parameters estimation. Analysis of gated PET images was performed with a previously validated 3-dimensional segmentation algorithm for identification of endocardial contours, enabling calculation of LV-volume [1]. This software program was validated in mice that underwent ^{18}F-FDG in comparison with the reference method MRI, assigned as the most optimal reference method. This validated software program was therefore used in the current rat study. The underestimation of LVEF by gated ^{13}N-NH_3 could be caused by partial volume effect and moderate uptake (count rate) of ^{13}N-NH_3 compared with ^{18}F-FDG. The ^{13}N positron has a longer range (1.4 mm) compared with the ^{18}F positron (1 mm). Another possible influence was the interval of one hour between ^{18}F-FDG and ^{13}N-NH_3 acquisition. Anaesthetics could also have influenced the results, due to difference in heart rate variability [10]. Rats with a myocardial infarction showed a lower LVEF, due to a higher ESV. Contour detection may be difficult in myocardial regions without perfusion. The contour may be drawn to widely. This will

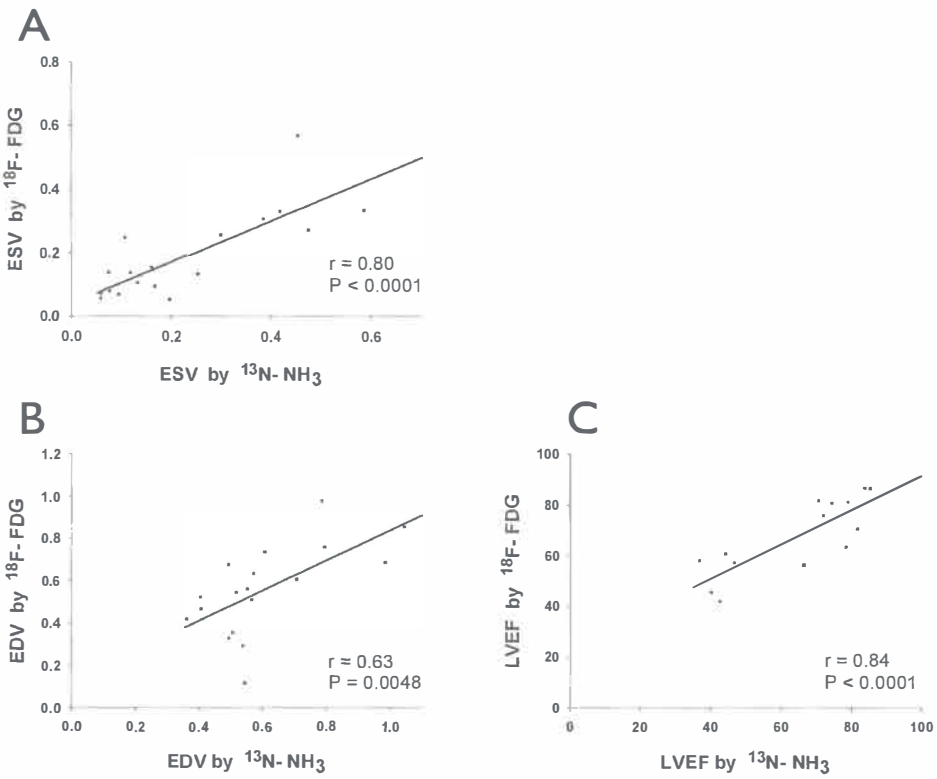

Figure 2. Correlations between ESV (A), EDV (B) and LVEF (C) determined with gated ^{13}N-NH$_3$ and ^{18}F-FDG PET.

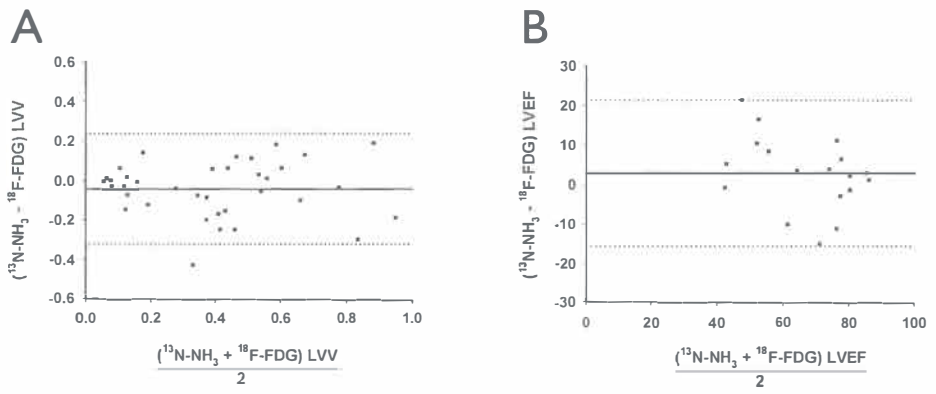

Figure 3. Bland Altman analysis of LV volumes (A) and LVEF (B) determined with gated ^{13}N-NH$_3$ and ^{18}F-FDG PET.

Table 3. Predictive performance of ^{13}N-NH_3 for LV volumes and LVEF measurements.

			Precision	Accuracy*		Bias	
	Mean	Min/Max	R			Mean	± 2 SD
ESV (ml)				0.05	0.1		
^{18}F-FDG	0.19 ± 0.14	0.05 - 0.57					
^{13}N-NH_3	0.23± 0.17	0.06 - 0.58	0.80	50	67	-0.04	-0.23/0.16
EDV (ml)				0.1	0.2		
^{18}F-FDG	0.56 ± 0.21	0.12 - 0.98					
^{13}N-NH_3	0.60 ± 0.19	0.36 - 1.04	0.63	44	83	-0.04	-0.38/0.29
LVEF (%)				5	10		
^{18}F-FDG	68.3 ± 13.7	42.0 - 86.8					
^{13}N-NH_3	65.4 ± 17.0	36.8 - 85.5	0.84	50	72	3	-15/21

** Percentage within specified range of gated ^{18}F-FDG*

result in higher ESV. Dedicated software programs will be needed to estimate the contour of regions with absent perfusion in the myocardial wall. Even though ^{13}N-NH_3 images rendered a lower LVEF and higher ESV and EDV, ^{13}N-NH_3 enables determination of ESV, EDV and LVEF with a satisfying accuracy when limits of agreement were set at 0.1 ml (ESV) 0.2 ml (EDV) and 10% (LVEF). These limits of agreement were identified after evaluation of intra-individual differences in 9 control animals.

Previous studies in humans with gated ^{13}N-NH_3 for the measurement LV function showed also higher values for EDV and ESV when compared to their reference method [5,6]. Khorsand et. al., found excellent agreement between values obtained with ^{18}F-FDG and ^{13}N-NH_3 in patients with coronary artery disease (CAD) However, they did not include healthy controls in their study [5].

Evaluation of LV function and volumes provide important prognostic information in CAD [4,11]. Combined measurement of cardiac function and perfusion in tomographic imaging will be a powerful method to monitor in a non invasively way the progress of the underlying disease and effect of treatments. In clinical practice, ^{13}N-NH_3 is an established method for the quantitative imaging of myocardial blood flow at rest and during pharmacologic stress to measure coronary flow reserve for the evaluation the significance of CAD[4,12] as well as for the detection of microvascular disease [13].

^{18}F-FDG has been proven to be a reliable tracer for the quantification of LV-function [1,2,14,15] and was therefore used as the reference method in this study. ^{18}F-FDG derived values for EDV, ESV and LVEF were compared with values derived from the golden standard MRI and showed an excellent agreement in small animals [1,16]. ^{13}N-NH_3 imaging overestimated

volumes compared to FDG imaging however, which suggests that there is considerably more overestimation of volumes by NH3 compared to MRI, as was evaluated in our previous study [1].

Small-animal PET scanners are becoming increasingly popular tools for biomedical research. The ability to sequentially monitor the same animal over time, assess biochemical processes in vivo, and demonstrate the effect of a new drug or the expression of membrane receptors [17] has brought the once-limited field of micro-PET and micro-SPECT [18] to the forefront of molecular medicine imaging. Our results indicate that micro-PET can be used to assess the function and geometry of left ventricle in both healthy rats and rats after myocardial infarction, which could possible lead to better monitoring of the progression of heart failure and the efficacy of multiple treatment strategies.

Study limitations

To mimic the clinical environment rats with and without myocardial infarctions were used to obtain functional values over a wide range. A potential pitfall in this setting is the delineation of the LV contours extended infarcted myocardial areas, which is more difficult and may lead to incorrect estimation of the LV volumes. Our program was able to estimate the contour of the LV properly in infarcted rats and systematic errors were reduced this way.

Conclusion

ECG-gated ^{13}N-NH_3 micro-PET correlates with gated ^{18}F-FDG for the assessment of LVV and LVEF in rats. Whereas LV volumes and LVEF in healthy rats can be measured reproducible with both approaches, LVEF in MI model was slightly underestimated with gated ^{13}N-NH_3 micro-PET. The small systematic bias and the limits of agreement are reasonable small enough to justify the conclusion that these methods can be used interchangeably. Dynamic ECG-gated ^{13}N-NH_3 PET may provide a novel non-invasive method to monitor parameters of cardiac function over time in healthy and heart failure animal models.

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Chapter 6

Imaging of cardiac and renal perfusion in rat models with ^{13}N-NH_3 micro-PET

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Submitted

Abstract

Purpose. Cardiac dysfunction leads to decreased organ perfusion. We aimed to measure cardiac and renal perfusion simultaneously with the use of ^{13}N-NH_3 micro-PET in different rat models.

Procedures. Ten male Wistar rats underwent sham surgery (n=5) or coronary artery ligation to induce myocardial infarction (MI, n=5). Eleven weeks later ^{13}N-NH_3 micro-PET scan was performed to study the cardiac and renal perfusion.

Results. Cardiac perfusion was significantly reduced in MI group and correlated with ejection fraction and MI size ($r=0.89$; $p<0.001$ and $r=-0.86$; $p<0.001$ respectively). Renal perfusion showed a notional 17% reduction in MI group when compared to sham (3.44 ± 0.40 vs 4.12 ± 0.48 ml/g/min resp.; NS). There was a trend towards greater reduction of perfusion in cortical than medullar region. Cortex perfusion was negatively correlated with histological changes.

Conclusion. ^{13}N-NH_3 micro-PET may be a potential tool for evaluation of cardiac and renal functional and perfusion changes in presence of cardiac dysfunction in rat models.

Introduction

Renal dysfunction is one of the most common co-morbidities in heart failure (HF) population. The co-existence of these entities results in worse prognosis [1]. These observations revived interest in the pathophysiological interrelationship between heart and kidney. Although the exact mechanisms remain unclear, there are multiple factors known that play a role in this interaction [2]. One of the pivotal mechanisms underlying this inter-organ relationship are the hemodynamic changes – reduced cardiac output leading to reduced renal blood flow (RBF). Reduced RBF has been shown to be the main determinant of renal function [3], which is a strong prognostic factor in HF population [1]. Not only is the total renal perfusion reduced in the presence of cardiac dysfunction, but also the differences in regional cortical and medullar blood flow have been observed [4]. Moreover, different mechanisms underlie the regulation of blood flow in cortical and medullar regions [5]. As a result, many vasoactive regulatory factors yield different effects on cortical and medullar blood flow. Since many of these factors, such as noradrenaline, angiotensin II, nitric oxide, are known to play role in cardiorenal syndrome and are being targeted by common treatment strategies, studies on regional renal perfusion in the presence of cardiac dysfunction might help understand better the pathophysiology behind this syndrome and improve the treatment strategies. Therefore a noninvasive, preclinical method for the evaluation of cardiac and renal kidney perfusion is needed. Positron emission tomography (PET) offers a promising, quantitative and minimally invasive alternative for measurements of organ perfusion. PET scans with ^{13}N-NH_3 have been used to evaluate cardiac perfusion [6, 7] as well as renal perfusion in humans [8] and big mammals [9, 10]. Since most of the experimental research on cardiorenal interaction is performed in rodents [11], we aimed to use the ^{13}N-NH_3 micro-PET to investigate the cardiac and renal perfusion in a healthy rat and in a rat model based on myocardial infarction.

Materials and methods

Animals

The study was performed in 10 male Wistar rats (Harlan, The Netherlands). Animals were housed in groups under standard conditions at 12h light/dark cycle until they reached 12 weeks of age. All animals received standard diet and water ad libitum during the study. At 12 weeks of age rats were subjected to myocardial infarction (MI) (n=5) by coronary artery ligation or sham surgery (n=5) as described before [12]. Approximately 11 weeks after the surgery, the rats were subjected to micro-PET scanning. One week after the PET scanning, the animals were sacrificed and heart and kidney tissue has been collected for further histological analysis. All animal experiments were performed by licensed investigators in compliance with the Law on Animal Experiments of The Netherlands. The protocol was approved by the Committee on

Animal Ethics of the University of Groningen.

Echocardiography

Echocardiographical measurements were obtained with the use of a Vivid 7 (GE Healthcare) equipped with a 10-MHz transducer. Under isoflurane anaesthesia rats were placed on a heating pad to maintain body temperature. The ejection fraction (EF) was calculated using the Teichholz method from the short axis images obtained at mid-papillary level. The measurement have been performed on 5 consecutive heart beats and presented as the mean of these values to avoid beat-to-beat variation.

Micro-PET data Acquisition

All rats were scanned while under 1.5% isoflurane anaesthesia and maintained in a fixed supine position. A heating pad was used to maintain a constant body temperature. ^{13}N-ammonia (^{13}N-NH_3) was injected intravenously into the penile vein. The radiotracer was administered as a 0.4 - 0.6 mL bolus, with an average dose of 55,2 ± 5,0 MBq. The camera (micro-PET Focus 220; Siemens/Concorde, TN, USA) was started simultaneously with injection of the tracer. Thereafter, a microCT scan was performed using a microCAT II system (Siemens Preclinical Solutions, Knoxville, TN, USA) using the same fixed bed with maintained stereotactic position. Micro-PET images were corrected for scatter and reconstructed applying an interactive reconstruction algorithm (OSEM 2D).

Micro-PET data processing

A 10 minutes list-mode acquisition protocol was used for ^{13}N-NH_3 imaging. PET data was reconstructed into 22 frames with a total of 10 min (12x5sec, 8x30sec, 2x150sec). Blood flow maps were analyzed manually by ROI analysis using Inveon Research Workplace software (Siemens; USA). The ROIs for cardiac perfusion evaluation were drawn on images in transverse plane on all images were heart was visible and included also infarcted areas (figure 1). The ROIs for renal perfusion evaluation were drawn on each kidney on 5 consecutive slides in coronal plane representing the middle section of the kidney. Firstly, the first 10 frames (5-60 seconds) were summed and the ROI for cortex region has been drawn (excluding the medulla and collecting system; figure 2A). Subsequently all frames were summed and the ROI for medullary region has been drawn (excluding collecting system and previously drawn cortical region, figure 2B). Finally, ROIs were pooled to assess the mean RBF. Due to the partial tissue trapping of ^{13}N-NH_3, a two-compartment kinetic model has been used as described before [10]. The arterial input function was determined from a ROI placed in the left ventricle of the heart.
All scans have been analysed by two independent researchers blinded for the groups. The mean values of these analyses are reported.

Histology

After collection kidneys were longitudinal bisected, fixed in formaldehyde solution and subsequently embedded in paraffin. Sections of 4 µm were stained with periodic acid Schiff (PAS) and scored on presence and degree of focal glomerular sclerosis as described before. FGS score was expressed in arbitrary units (AU) as described before [13]. The sections were evaluated by the examiner who was blinded for the groups.

The heart was dissected, cooled in ice-cold saline for diastolic arrest. A midventricular slice was embedded in paraffin and subsequently stained with Sirius Red/ Fast Green. The infarct size was expressed as the mean of the inner and outer percentage of scar tissue to the inner and outer circumference of the left ventricle as described before [14].

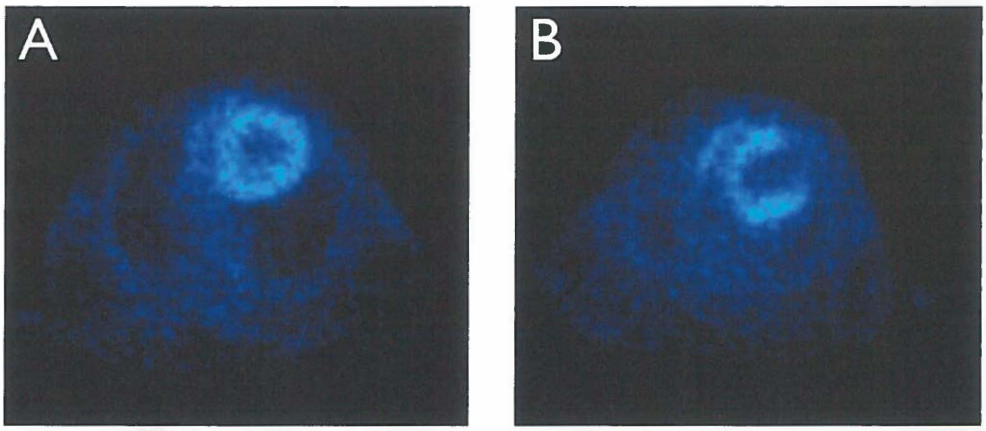

Figure 1. *Analysis of cardiac perfusion.* ***A:*** *sham group;* ***B:*** *MI group*

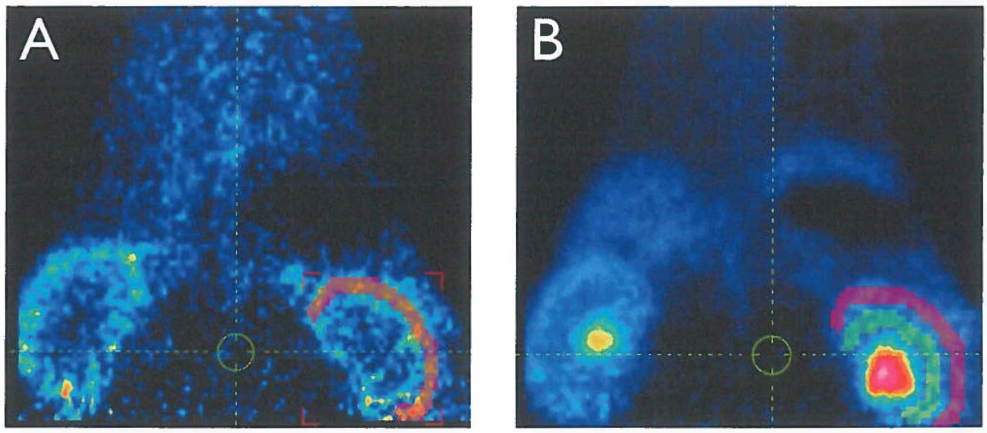

Figure 2. *Analysis of regional renal blood flow. The placement of ROIs for perfusion analysis.* ***A:*** *selection of cortical region;* ***B:*** *selection of medullar region*

Statistical analyses

All data is presented as mean ± SEM. A Mann-Whitney U test was used to compare the results between the groups. Differences were considered significant at the level of 0.05.
All analyses were performed with the use of PASW software version 18.

Results

At the moment of termination, mean body weight was similar in both groups (458 ± 19 vs. 459 ± 20 g for MI and sham group respectively). The mean LVEF was 55.8 ± 4.1 % and 77.8 ± 1.0% for MI and sham respectively ($p = 0.008$). The mean infarct size was 32 ± 8 % in the MI group ($p=0.008$ vs. sham). There was a strong correlation between LVEF and MI size in the whole study group ($r = -0.89$; $p<0.001$).
Cardiac perfusion measured with ^{13}N-NH_3 micro-PET was significantly reduced in MI group in comparison with the sham group (6.32 ±0.96 vs. 11.55±0.61 respectively). There was significant correlation between perfusion obtained with micro-PET scan and both LVEF ($r = 0.89$; $p < 0.001$) and MI size ($r = -0.86$; $p < 0.001$).
Renal global and regional perfusion measurements with ^{13}N-NH_3 micro-PET showed no statistically significant differences between the left and right kidney in any of the groups (table 1). Mean renal perfusion was decreased by 17% in MI group in comparison to sham, however the difference was not significant ($p=0.42$). Similar pattern has been seen with regard to regional perfusion – both cortex and medulla perfusion values were lower in MI group, however not significantly. When compared to values obtained from the sham group, the reduction of the regional perfusion in MI group seemed to be greater in cortex than in medulla (22% vs. 9% respectively), resulting in increased ratio between medulla and cortex perfusion (0.68 ± 0.04 vs. 0.57 ± 0.05), however this increase did not reach statistical significance. There was no significant correlation between this ratio and any of the cardiac parameters.
The mean renal perfusion showed positive correlation with cardiac perfusion ($r = 0.65$; $p = 0.042$) (figure 3A), but not with LVEF. There was significant correlation between cortical and cardiac perfusion ($r = 0.73$; $p = 0.016$) (figure 3B) and a trend towards correlation with LVEF ($r = 0.62$; $p = 0.058$) and MI size ($r = -0.60$; $p = 0.070$). There were no significant correlations or trends found for medullar perfusion with any of the cardiac parameters. The histological analysis showed increased focal glomerulosclerosis in MI group (5.4 ± 1.6 AU vs 1.0 ± 0.4 AU for MI and sham, respectively; $p = 0.016$) (figure 4). There was a negative correlation between FGS and both total renal and cortical perfusion ($r = -0.67$, $p = 0.035$; $r = -0.66$, $p = 0.036$ resp.) (figure 3C and 3D).

Table 1. *Regional and total kidney perfusion measurements. Data expressed in ml/g/min and presented as mean ± SEM.*

	MI group (n=5)	Sham group (n=5)
Total kidney perfusion – mean	3.44 ± 0.40	4.12 ± 0.48
Left kidney	3.41 ± 0.42	4.06 ± 0.51
Right kidney	3.46 ± 0.39	4.17 ± 0.46
Right/left kidney ratio	1.02 ±0.02	1.05 ± 0.06
Cortex perfusion – mean	3.85 ± 0.37	4.92 ± 0.59
Left cortex	3.80 ± 0.39	4.88 ± 0.63
Right cortex	3.89 ± 0.36	4.97 ± 0.57
Right/left cortex ratio	1.03 ± 0.03	1.03 ± 0.06
Total medulla perfusion – mean	2.65 ± 0.36	2.90± 0.52
Left medulla	2.64 ± 0.35	2.98 ± 0.58
Right medulla	2.66 ± 0.38	2.82 ± 0.47
Right/left medulla ratio	1.00 ± 0.03	1.00 ± 0.09

Discussion

In the present study, we used ^{13}N-NH_3 micro-PET to evaluate the cardiac and renal perfusion in healthy rats and rats with myocardial infarction. We showed that experimental cardiac dysfunction leads to reduced cardiac perfusion and we found a trend towards reduction in renal perfusion. The latter reduction seemed to be higher in the cortical than medullar region of the kidney. Cardiac perfusion was significantly correlated with renal and cortical, but not with medullar perfusion. Interestingly, there was a negative correlation between cortical perfusion and the severity of histological changes in this region.

Myocardial infarction leads often to reduction in cardiac function and subsequently reduced peripheral organ perfusion. In our study, experimental MI led to decrease in cardiac perfusion. Measured cardiac perfusion was strongly correlated with infarct size and LVEF. There was a trend towards lowering of renal perfusion in animals with experimentally induced cardiac dysfunction; however the difference did not meet the statistical significance. The lack of significance might have resulted from small experimental groups and some variation in the induced infarct size – it has been shown that small infarcts do not lead to substantial reduction in cardiac function and cardiac output therefore the renal perfusion might not be significantly affected.

Not only is the total renal perfusion affected in the presence of cardiac dysfunction, but it

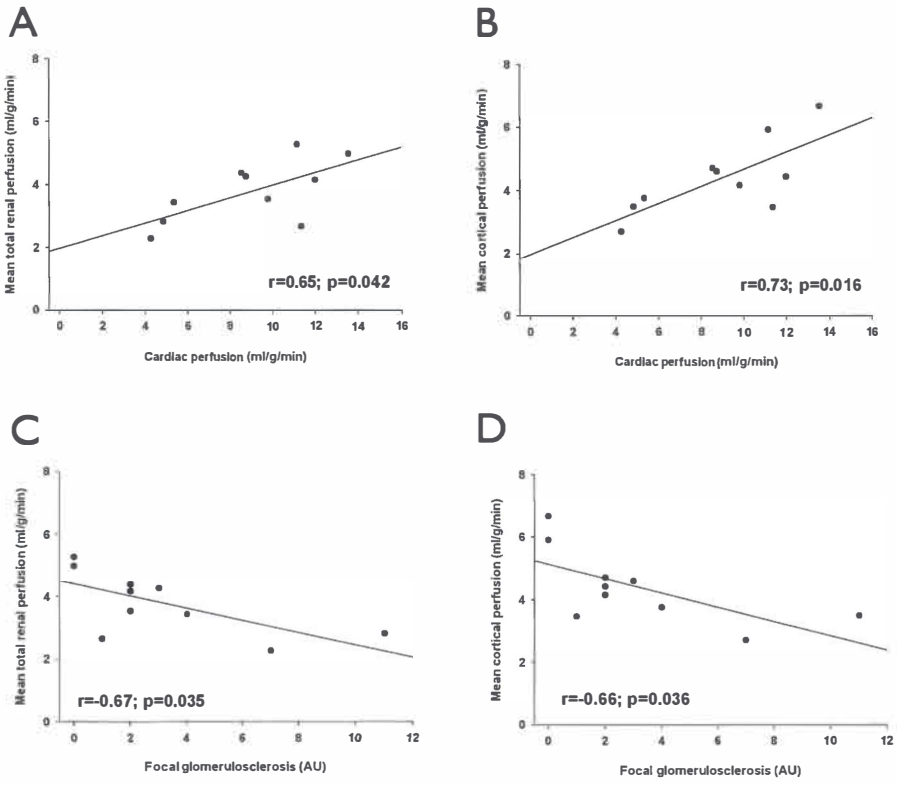

Figure 3. *Correlations between total renal and cardiac perfusion (**A**), cortical and cardiac perfusion (**B**); total renal perfusion and focal glomerulosclerosis (**C**); cortical perfusion and focal glomerulosclerosis (**D**). AU; arbitrary units.*

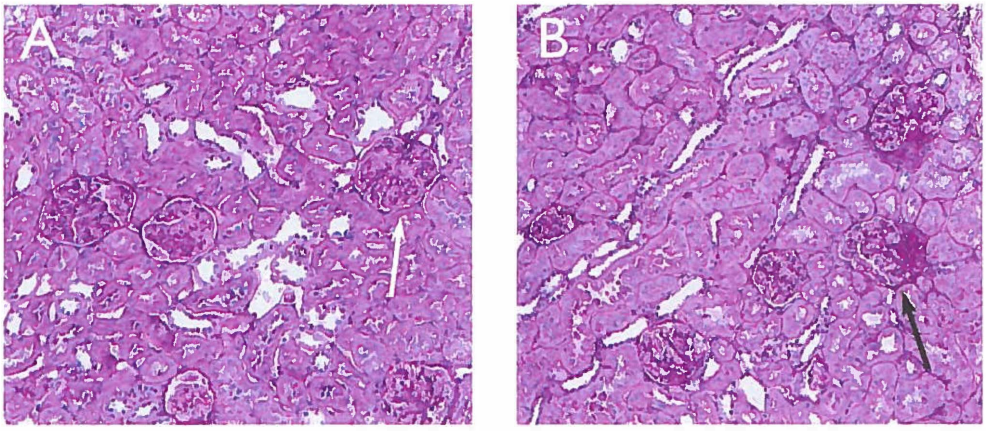

Figure 4. *The representative photographs of cortex region. **A**: sham group; **B**: MI group; white arrow indicating normal glomeruli, black arrow - sclerotic glomeruli.*

has also been shown, that the cortical blood flow is markedly diminished, whereas medullary blood flow appears to be preserved [4]. We were able to show this trend in present analysis. Although the differences were not significant, the cortical blood flow was reduced by 22% in comparison to 9% reduction in medullar perfusion. Significant correlation between cardiac and cortical but not medullar perfusion found in our study supports the observations of different regulation mechanisms of regional renal blood flow [5]. Whereas in the medullar region vasodilation occurs to preserve the blood flow [15], the activated renin-angiotensin-system leads to vasoconstriction in the cortical region[16]. Increased angiotensin II levels lead to glomerular hyperperfusion and increased filtration fraction and preservation of kidney function. However, on long term angiotensin II causes glomerular matrix accumulation and progression of glomerulosclerosis. Activation of regulatory systems and preferential vasoconstriction of renal cortical vessels may also contribute to the increased sodium and retention and subsequently increased venous pressures and edema formation[16]. This leads to higher pressures in the kidney and hypoxia, which may cause further histological changes and decrease of kidney function [17]. In the present study we showed increased glomerulosclerosis in MI group and a significant correlation between severity of glomerulosclerosis and cortical perfusion. Again, this correlation was not found for the medullar perfusion which provides additional evidence for different regulatory mechanisms.

In the present study, we used the ^{13}N-NH_3 micro-PET for cardiac and renal perfusion measurement in rats. The advantage of this approach is the possibility of simultaneous measurement of perfusion of two organs. Recently, we have shown that ECG-gated ^{13}N-NH_3 micro-PET can be also used for estimation of cardiac volumes and function, which extends the possibilities of this method [18]. PET imaging furthermore allows repetitive imaging due to its non-invasive character. Whereas cardiac perfusion measurements have been described before in literature [6, 7], regional renal perfusion measurements remain still challenging. There have been discrepancies reported between various methods [19] and even the measurements with radioactive microspheres have been shown to have its limitations in RBF determination [19]. The technique we used might offer a promising, quantitative and minimally invasive alternative for measurements of regional RBF [20].

The accuracy of the RBF measurements with the use of PET depends on the tracer. We used the protocol with ^{13}N-NH_3, which has been described before and validated by comparison to microspheres study [10]. Renal retention of ^{13}N and its relative long half-life compared with ^{15}O-water allows extended image acquisition periods. Also the shorter range of the ^{13}N positron compared with ^{15}O-water is attractive. This leads to better quality of acquired images. Because of the tracer kinetics, this method requires a two-compartment model. Since ^{13}N-NH_3 is rapidly metabolized, it has been argued that the values of arterial input should be corrected for ^{13}N-metabolites in blood. Analysis by Chen showed that there is only little contamination, which leads to limited underestimation of the results and therefore the correction is not necessary [10].

Due of the complicated structure of the kidney, systematic errors due to finite spatial resolution and partial-volume effects might occur. This might lead to underestimation of cortical flow and thus the ability to show subtle differences in regional blood flow might be limited. It cannot be also excluded that within the cortical ROIs an unknown part of medullar region has been included and vice-versa. Although the maximal size of ROIs did correspond with the size of the cortex region found in histological studies and the PET flow maps provided relatively sharp boundaries of cortical region, the accuracy of the ROIs location should be interpreted with some caution.

Our study is limited by the small size of experimental groups. There was some variation in infarct size between animals in MI group, which in some animals might have resulted only in a slight reduction of cardiac function and therefore limited effects on renal perfusion. We did not perform the simultaneous microsphere blood flow measurements to compare the results; however the used protocol has been described and validated in literature before.

Conclusion

Micro-PET with the use of ^{13}N-NH_3 might serve as an useful non-invasive tool for measurement of cardiac and regional renal perfusion in small animal models. The possibility to evaluate regional renal perfusion in rodents might help to understand the changes occurring in kidney in the presence of cardiac dysfunction. The use of ^{13}N-NH_3 allows simultaneous assessment of cardiac and renal perfusion in rodents. This all may contribute to better understanding of the cardiorenal syndrome.

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Chapter 7

Prognostic value of renin and prorenin in heart failure patients with decreased kidney function

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Abstract

Background. The renin-angiotensin-aldosterone system (RAAS) plays a key role in the progression of heart failure (HF) and concomitant kidney dysfunction. Despite the use of RAAS blockade, sustained activation of RAAS has been suggested to link with adverse outcome. We aimed to investigate the prognostic value of active plasma renin concentration (APRC) and prorenin in patients with HF treated with RAAS-blocking agents and its relationship with kidney function parameters.

Methods. One hundred clinically stable patients with HF, treated with RAAS-blocking agents, were studied. Renal function parameters including effective renal plasma flow and glomerular filtration rate were measured invasively. The combined end point consisted of all-cause mortality, heart transplantation, and admission to hospital for HF.

Results. Mean age was 58 ± 12 years, and 76% were men. Mean left ventricular ejection fraction was 28 ± 9, and median APRC levels were 24.3 ng/mL per hour. Active plasma renin concentration was most strongly associated with mean arterial pressure ($r = 0.60$, $P < 0.001$). In multivariate linear regression analysis, age, mean arterial pressure, angiotensin II concentration, and use of aldosterone antagonists were significantly related with APRC (adjusted $R^2=0.53$). Patients in the highest quartile of APRC had a worse prognosis. In multivariate analysis, APRC remained associated with worse prognosis: HR 2.87 (95% CI 1.14-7.20), $P = 0.025$. Prorenin did not show prognostic value. The prognostic value of APRC was strongest in patients with decreased kidney function.

Conclusions. Our data indicate that APRC is a strong prognostic factor in patients with HF in the presence of RAAS inhibition, especially in patients with kidney dysfunction.

Introduction

Heart failure (HF) is a complex syndrome associated with substantial mortality [1]. Decreased perfusion activates multiple compensatory mechanisms, of which the renin-angiotensin-aldosterone system (RAAS) is widely acknowledged to be of special importance [2]. Activation of the RAAS cascade leads to increased blood pressure and water and salt retention, which upholds perfusion. Although initially aimed to preserve cardiovascular homeostasis, long-term activation of the RAAS eventually has an adverse effect on cardiac structure and function leading to progressive remodeling and ultimately worsening HF.

Many patients with HF have compromised kidney function, which is associated with a worse prognosis [3,4]. Decrease in cardiac output and/or increased venous pressure in HF result in decreased renal blood flow, which has been shown to be the most important determinant of renal function in patients with HF [5,6]. Present therapies of HF cannot fully prevent the development of progressive HF and further deterioration of the renal function. Strategies to block the RAAS, for instance with ACE inhibitors (ACEi), angiotensin II receptor blockers (ARBs), and aldosterone receptor antagonists (ARAs), have been proven to reduce HF centered morbidity and mortality [7-10]. However, as a result of lack of negative feedback, RAAS-blocking agents increase plasma renin concentration and plasma renin activity (PRA). Although initially deemed unharmful, sustained increased levels of PRA have been found to be a strong and independent prognostic factor in patients with HF [11,12]. Little is known about the prognostic value of active renin concentration in the presence of RAAS inhibition [13]. Therefore, efficacy of RAAS blockade, single or dual or triple, and its effect on neurohormonal status are subject to study, both in HF without and with concomitant renal disease.

Recently, it has been shown that prorenin not only serves as a renin precursor but also has its own function. It has been suggested that it acts by generating angiotensin I (AngI), as well as independently of AngI generation [14]. Taking into account much higher prorenin levels than renin levels, further investigation of the relation between renin and prorenin could be of special interest to better understand the pathophysiologic role of RAAS in HF [15].

The aim of the current study was to evaluate the prognostic value of active plasma renin concentration (APRC) in a group of patients with chronic HF being treated with RAAS-blocking agents. Second, we tried to determine to what extent this value is dependent on prorenin concentrations and whether prorenin has individual prognostic value or adds to prognostic value of APRC in this population. Finally, we tried to determine the interrelationship of increased levels of APRC with other parameters of indices of renal impairment on clinical outcome in this group of patients.

Methods

Study design

The main study design has been published elsewhere [16]. In short, outpatients with HF, ≥18 years old, with left ventricular ejection fraction (LVEF) <45%, and clinically stable were asked to participate. All patients used RAAS inhibitors (ACEi, ARBs, ARAs, or a combination), and all medication had to be stable for at least 1 month. Exclusion criteria were a myocardial infarction within the last 3 months, cardiac surgery or angioplasty within the last 3 months or scheduled to undergo these procedures, unstable angina pectoris, primary renal disease, prior organ transplantation, or chronic use of renal function–compromising medication. Baseline measurements included weight, height, blood pressure (mean arterial pressure [MAP] was calculated as follows: [2 x diastolic blood pressure + 1 x systolic blood pressure]/3), and assessment of New York Heart Association function class. All patients underwent measurements of renal function. Glomerular filtration rate (GFR), effective renal plasma flow (ERPF), and extracellular volume were measured by the clearances of 125I-iothalamate and 131I-hippuran as described earlier [17]. The filtration fraction (FF) was calculated as the ratio of GFR and ERPF and expressed as percentage. The study protocol was approved by the institutional ethics committee. All patients gave written consent.

Active plasma renin concentration, angiotensin II (AngII), and prorenin concentrations were measured as markers for RAAS activity. Active plasma renin concentration was measured by quantifying AngI generation in the presence of excess exogenous angiotensinogen, obtained from nephrectomized sheep as described before in detail,18,19 and expressed as ngAngI/mL per hour. The detection limit was 0.1 ngAngI/mL per hour. Angiotensin II was measured by a specific radioimmunoassay after extraction from plasma by reversible adsorption to octadecylsilyl silica (Sep-Pak; Waters, Milford, MA) as described before [20]. Using this method, the cross-reactivity with both AngI and Ang (1-7) was low (<0.2%). Prorenin was measured with Human Prorenin ELISA kit (Innovative Research, Novi, MI) with prorenin-depleted human plasma and prorenin-depleted human plasma spiked with recombinant human prorenin from human embryonic kidney cell culture used as standards. The detection limit was 0.01 ng/mL. Analyses were performed in a routine setting according to the guidelines of the manufacturer. N-terminal pro–brain natriuretic peptide (NT-proBNP) was determined by electrochemiluminescence assay (Roche Diagnostics, Mannheim, Germany).

High-sensitive C-reactive protein was measured by nephelometry with a threshold of 0.156 mg/L (BNII N, Dade Bahring, Marburg, Germany). C-reactive protein levels below the detection level were scored as 0.156 mg/L.

Urinary albumin excretion rate (in milligrams per 24 hours) was determined as a parameter of structural kidney damage and measured by nephelometry (Dade Behring Diagnostics, Marburg, Germany).

Follow-up

Patients' follow-up started after renal function measurements had taken place. The primary (combined) end point consisted of all-cause mortality, heart transplantation, or admission to hospital for HF.

Relation between APRC and GFR

To evaluate the relation between APRC levels and kidney function on prognosis, the study population was divided into APRC groups above or below the median with preserved renal function (≥60 mL/min/1.73 m2) or impaired renal function (<60 mL/min/1.73 m2), creating 4 subgroups.

Statistical analysis

Data of variables are presented as mean ± SD when normally distributed, medians with interquartile ranges when non-normally distributed, and frequencies and percentages for categorical variables. In the linear regression analysis, non-normally distributed data were log transformed. Multivariate linear regression analysis, including all univariate associated variables, was used to determine the contribution of different variables to APRC. P = 0.10 was used for removal of nonsignificant variables. Cox proportional hazards regression analysis was used to evaluate the association with baseline parameters and the occurrence of end points. Continuous variables were divided by their SD to allow comparison of relative contributions of different variables. Statistical analyses were performed using SPSS version 16.0 (SPSS Inc, Chicago, IL).

The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the manuscript, and its final content.

Results

The study population consisted mostly of male patients (76.5%) with a mean age of 58 ± 12 years. All patients received RAAS inhibition (88% ACEi, 14% ARBs, and 36% ARAs) and most were treated with beta-blockers (84%) and diuretics (70%). Mean LVEF was 28% ± 9%, and mean GFR was 75 ± 27 mL/min/1.73 m2. Median APRC levels were 24.3 ng/mL per hour. We classified patients according to quartiles of APRC (table 1). Patients in the highest quartile were more often female and had higher levels of prorenin, AngII, increased creatinine levels, lower values of GFR and ERPF, and more severe HF as represented by lower LVEF, lower MAP, and higher NT-proBNP levels. They were also more often treated with diuretics and ARAs. There was no difference between the groups in use of ACEi or ARBs, neither in percentage nor in dose.

Table 1. Baseline characteristics for study population and according to APRC quartiles.

Variables	Total population	APRC quartile				P-value
		1	2	3	4	
n	100	25	25	25	25	
Age (years)	58 ± 12	63 ± 10	58 ± 12	55 ± 11	56± 12	0.015
Male sex (n, %)	76 (76)	21 (84)	19 (76)	19 (76)	17 (68)	0.211
LVEF (%)	28 ± 9	32 ± 8	27 ± 9	27 ± 8	25 ± 11	0.024
NYHA class, %						<0.001
I	14	24	12	20	0	
II	46	52	68	40	24	
III	30	24	16	28	56	
IV	10	0	4	12	20	
Hemoglobin levels (g/dL)	14,1 ± 1,4	14,4 ± 1.1	13,9 ± 1,5	14,1 ± 1,2	13,8 ± 1,7	0.227
Sodium (mmol/L)	136 ± 3	137 ± 4	136 ± 2	136 ± 3	134 ± 3	0.014
ECV (L/kg BW)	0.26±0.05	0.26±0.04	0.28±0.05	0.25±0.04	0.25±0.05	0.464
Blood pressure						
SBP (mmHg)	120 ± 21	136 ± 19	124 ± 15	114 ± 18	104 ± 16	<0.001
DBP (mmHg)	69 ± 12	77 ± 10	71 ± 12	67 ± 9	60 ± 10	<0.001
MAP (mmHg)	86 ± 14	97 ± 11	89 ± 12	83 ±11	75 ± 10	<0.001
Renal function						
Serum creatinine (mmol/dL)	112 ± 34	105 ± 23	99 ± 23	119 ± 38	126 ± 43	0.006
GFR (ml/ min/1.73m2)	75 ± 27	84 ± 26	83 ± 20	74 ± 30	60 ± 25	<0.001
ERPF (ml/ min/1.73m2)	275 ± 88	288 ± 87	303 ± 77	271± 100	239 ± 75	0.020
FF (%)	27 ± 5	29 ± 4	28 ± 4	27 ± 5	25 ± 7	0.002
UAE (mg/day)	5,70 (3,45-11,10)	5,95 (4,2–8,6)	6,15 (2,86–12,25)	4,05 (3,0–12,95)	6,0 (3,9–11,1)	0.574
Neurohormones						
NT-proBNP (pg/ml)	612 (269–1784)	635 (343–967)	520 (259–1355)	491 (210–2007)	1004 (362–2076)	0.394
APRC (ng/ml/h)	24,3 (5,6-71,9)	2,6 (1,5–3,9)	10,9 (9,3–14,5)	42,1 (35,8–49,4)	121,2 (94,7–157,3)	<0.001

Table I continued

Variables	Total population	APRC quartile				P-value
		1	2	3	4	
Angiotensin II (pmol/l)	9,9 (4,7–15,1)	5,5 (3,1–9,8)	7,8 (4,2–11,3)	11,3 (9,6–19,1)	13,7 (10,0–19,9)	<0.001
Prorenin (ng/ml)	2,17 (1,30–3,89)	1,36 (0,90–2,10)	2,20 (1,12–3,61)	2,21 (1,64–4,02)	3,02 (2,04–4,61)	<0.001
hsCRP (mg/l)	2,5 (1,0– 4,3)	1,9 (0,8–3,8)	1,9 (0,8–5,5)	2,6 (1,4–3,9)	3,6 (0,9–5,5)	0.121
Treatment, n (%)						
ACEi	88 (88)	22 (88)	23 (92)	22 (88)	21 (84)	0.584
ARBs	14 (14)	4 (16)	2 (8)	4 (16)	4 (16)	0.798
ACEi + ARB	2 (2)	1 (4)	0 (0)	1 (4)	0 (0)	0.525
Beta-blockers	85 (85)	23 (92)	18 (72)	21 (84)	23 (92)	0.709
Diuretics	69 (69)	13 (52)	13 (52)	18 (72)	25 (100)	<0.001
Aldosterone antagonists	36 (36)	1 (4)	6 (24)	12 (48)	17 (68)	<0.001

LVEF: Left ventricular ejection fraction; NYHA: New York Heart Association; ECV: extracellular volume; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; GFR: glomerular filtration rate; FF filtration fraction; UAE: urinary albumin excretion; BNP: brain natriuretic peptide; APRC: active plasma renin concentration; ACEi: angiotensin converting enzyme inhibitors; ARBs: angiotensin receptor blockers; hsCRP: high-sensitive C-reactive protein. P value for trend.

Relationship of cardiorenal hemodynamic and functional parameters with APRC and prorenin

Active plasma renin concentration was most strongly associated with MAP ($r = -0.60$, $P < 0.001$). Age, renal hemodynamic (ERPF, FF), renal functional (GFR), and cardiac (LVEF) parameters were also significantly related to APRC. With respect to RAAS-related parameters, prorenin and AngII concentrations were significantly related to APRC. With respect to treatment, only diuretics and ARAs showed a relationship with APRC. In multivariate linear regression analysis (table 2), age, MAP, AngII concentration, and use of ARAs remained significantly related with APRC (adjusted $R^2 = 0.53$).

Because of high colinearity between renal function parameters ($r = 0.90$, $r = 0.73$ for GFR vs ERPF and creatinine, respectively), only GFR and FF were included in the multivariate model. Prorenin was significantly related to GFR, MAP, APRC, AngII concentration, and use of ARAs and diuretics. In contrast to APRC, there was only a modest borderline nonsignificant association with ERPF ($P = 0.054$), FF ($P = 0.054$), and plasma NT-proBNP ($P = 0.05$) and no association with age ($P = 0.580$), creatinine ($P = 0.08$), or LVEF ($P = 0.228$). In multivariate analysis, only

Table 2. Multivariate regression analysis for APRC

Variable	B	SE	Beta	p-value
Age	-0.026	0.011	-0.200	0.016
GFR	-0.010	0.005	-0.171	0.065
MAP	-0.044	0.009	-0.393	<0.001
Angiotensin II	0.326	0.130	0.193	0.014
Aldosterone antagonists	0.730	0.282	0.229	0.011
Adjusted R^2			0.530	<0.001

NT-proBNP, APRC, and use of diuretics remained significant predictors of prorenin (adjusted $R^2 = 0.26$).

Outcome related to APRC and prorenin

During a 3-year follow-up, 11 patients died, 5 underwent heart transplantation, and 12 were admitted to the hospital for congestive heart failure. Patients with the highest APRC had a worse prognosis in comparison with other groups (60% reached end point in quartile 4 in comparison with 20%, 12%, and 12% in quartiles 1, 2, and 3, respectively; P = 0.001) (figure 1). In a predefined multivariate Cox regression analysis, including age, gender, hemoglobin levels, parameters of renal and cardiac function, and treatment, only APRC, NT-proBNP, and hemoglobin levels remained significantly related to worse outcome (HR 2.87 [95% CI 1.14-7.20], P = 0.025; HR 3.37 [95% CI 1.47-7.71], P = 0.004; and HR 1.88 [95% CI 1.10-3.21], P = 0.022, respectively). Prorenin did not contribute significantly to prognosis in the multivariate analysis.

Active plasma renin concentration outcome association by GFR strata

As shown in table 3, patients with decreased kidney function and high APRC levels were of highest risk for poor clinical outcome. Considering subgroups with preserved kidney function, high levels of APRC did not change this risk. In contrast, in patients with decreased kidney function, high APRC levels were associated with worse prognosis with an HR of 3.54 (95% CI 1.02-12.36, P = 0.047). After adjustment for cardiac parameters (MAP, LVEF, and NT-pro-BNP), the point estimate of the HR remained numerically comparable; however, APRC lost its statistical significance: HR 3.10 (95% CI 0.82-11.76, P = 0.096). When subgroups with high APRC levels were taken into account, the presence of kidney dysfunction significantly increased the risk for worse outcome: HR 11.66 (95% CI 3.34-40.71, P < 0.001) and 5.92 (95% CI 1.58-22.21, P = 0.008) before and after adjustment, respectively. Kaplan-Meier survival curves are shown in figure 2.

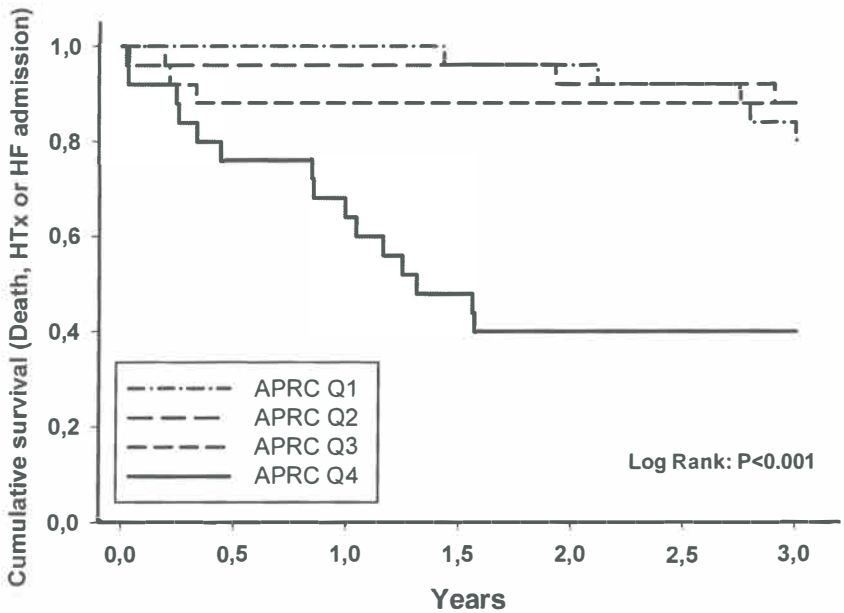

Figure 1. Survival analysis according to quartiles APRC. APRC Q1, Q2, Q3, Q4 indicate quartile 1, 2, 3, and 4, respectively. HTx, heart transplantation.

Finally, we also calculated the interaction term between APRC and GFR levels using both variables dichotomized according to the strategy mentioned above, and we found a trend toward interaction (P = 0.109).

Discussion

In the present analysis, we confirm that APRC is a strong prognostic factor in patients with HF treated with RAAS blocking agents. For the first time, we show that APRC is of prognostic value especially in HF patients with impaired kidney function. Even after adjustment for various kidney function parameters, which were measured invasively using state-of-the-art techniques, APRC remained a significant predictor of outcome. Next to APRC, NT-proBNP and hemoglobin levels were also of predictive value. Increased levels of active renin have previously been shown predictors of mortality in patients with HF [13,21]. Several explanations can be proposed for the observed prognostic value of high levels of APRC in HF. First, high APRC may reflect the severity of the cardiac disease [22] and/or kidney dysfunction [4]. However, in our population even after adjusting for various cardiac and renal parameters, APRC remained a significant

Table 3. APRC outcome association by GFR strata

	Unadjusted		Adjusted for cardiac function *	
	HR (95% CI)	P	HR (95% CI)	P
Subgroup 1 (n=42)	1		1	
Subgroup 2 (n=29)	0.77 (0.19-3.09)	0.714	0.68 (0.16-2.97)	0.610
Subgroup 3 (n=10)	2.54 (0.64-10.15)	0.188	1.30 (0.31-5.48)	0.720
Subgroup 4 (n=21)	9.00 (3.44-23.53)	<0.001	4.03 (1.25-13.00)	0.020

*Subgroup 1: GFR≥60 mL/min/1.73m2, APRC<24.3 ng/mL/h; Subgroup 2: GFR≥60 mL/min/1.73m2, APRC≥24.3 ng/mL/h; Subgroup 3: GFR<60 mL/min/1.73m2, APRC <24.3 ng/mL/h; Subgroup 4: GFR <60 mL/min/1.73 m2, APRC ≥24.3 ng/mL/h. * Adjusted for MAP, LVEF, and NT-proBNP.*

predictor of outcome, so that direct effects via APRC may explain its prognostic significance. Another possible explanation may be that higher APRC levels reflect more intensive RAAS inhibition. Interestingly, use of captopril eliminated the prognostic value of PRA on 1-year mortality in the SAVE trial [23]. However, this is in contrast to reports of the survival benefit of RAAS inhibition, especially in patients with the most neurohormonal response [7-9]. We also did not find significant differences in use of ACE inhibitors or ARBs nor in ACEi/ARBs dose between patients with different APRC levels.

Active plasma renin concentration levels were significantly related to age, MAP, and AngII concentration and the use of aldosterone antagonists, which confirms reports from the literature [13,24]. We did observe differences in the use of ARAs between patients with different APRC levels. Patients using aldosterone antagonist had higher APRC levels than those who did not use this treatment. However, survival analysis of the subgroup of patients using aldosterone antagonists showed that also in this group APRC levels were of prognostic value, with the lowest survival in the patients with highest levels of APRC (data not shown).

There were also differences in diuretics use between groups of patients in our study. Diuretics have been shown to increase renin secretion, mostly because of extracellular volume contraction and electrolyte disturbances that are being sensed by the macula densa apparatus [25]. Although we did not observe any differences in extracellular volume, there was a trend toward lower sodium concentration with increasing APRC, so we cannot fully exclude the effect of diuretics on APRC levels.

Our population was characterized by a substantial number of HF patients with decreased renal function and renal perfusion. We are the first to show that high APRC levels have strong prognostic value especially in patients with decreased kidney function. Because APRC levels

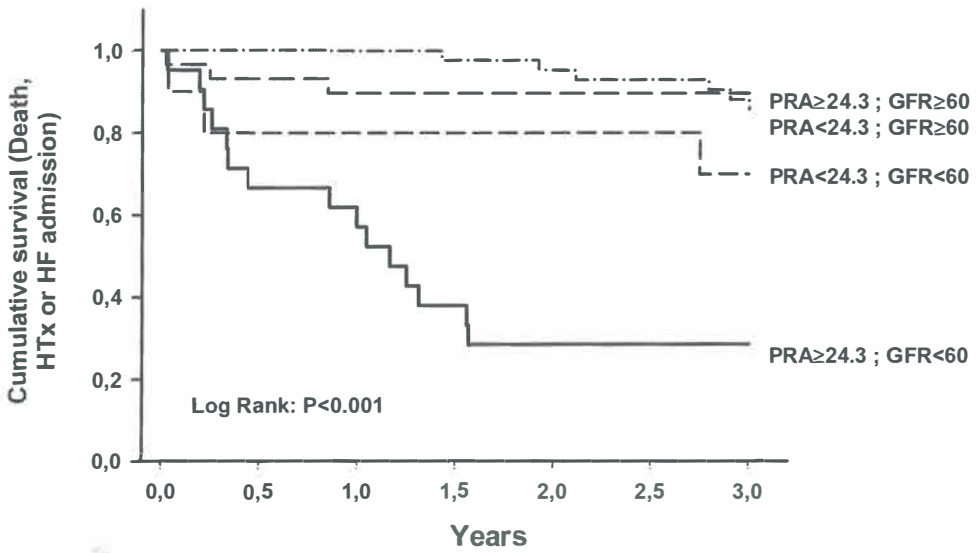

Figure 2. Kaplan-Meier curves according to baseline APRC levels and GFR.

may also reflect decreased renal perfusion [5] and, in HF, renal blood flow decrease is especially pronounced in cortical regions [26], the region responsible for active renin secretion [27] this may be of eminent importance.

What factors underlie the association of increased APRC and worse outcome? Renin and prorenin can bind to the recently cloned prorenin/renin receptor and cause the development of fibrosis and damage without AngII formation [14,15]. Experimental studies have shown that smooth muscle cell–specific overexpression of the prorenin/renin leads to systemic hypertension. Reversely, strategies aimed to block the prorenin/renin receptor using the handling region peptide could ameliorate hypertensive renal damage [28]. Whether renin and prorenin exert maladaptive signaling in HF is still subject to study, and we cannot exclude this effect in our study. As expected, we observed a correlation between APRC and prorenin concentration. However, after adjustment for multiple variables, prorenin did not show prognostic value in our population, which questions diagnostic use of prorenin.

Another possibility to consider is the ACE escape phenomenon. It has been shown that because of the existence of alternative pathways, AngII formation is not completely blocked upon ACE inhibition [29-31]. Moreover, the use of ARAs on top of ACE inhibition induces further activation of RAAS system and leads to increased levels of active renin and to increased AngII formation. We were able to confirm those findings in our population; patients with highest APRC levels were more often using ARAs and had also highest AngII levels.

Furthermore, activation of the sympathetic nervous system (SNS), reported in HF, leads to

increased secretion of renin [32,33]. Blockade of SNS with beta-blockers reduces APRC in HF, also in presence of ACEi [34,35]. We cannot fully exclude the hypothesis that association of APRC with the outcome is related to SNS activation in our patients; however, that there was no significant differences in beta- blockers use between groups of patients with different APRC and that prescription of those drugs does not influence the prognostic value of renin [21] make this hypothesis unlikely.

Clinical implications

Blocking RAAS activity with ACEi and/or ARBs became a common practice in the last 2 decades. This treatment has resulted in reduced mortality in this group of patients [7]. However, it has previously been shown [13] and confirmed in the current study that increased APRC levels correlate with worse outcome, also in the presence of RAAS blockade. The prognostic value of the APRC was strongest in the group of patients with impaired kidney function. This suggests that a treatment strategy including drugs blocking renin might be more beneficial in this group of patients. Direct renin inhibitors showed to have favorable effects on neurohormonal status in patients with HF [36] and thus may be a promising treatment in patients with HF, especially in the presence of kidney dysfunction. However, we have to await mortality trials for definite proof.

Study limitations

Our study is limited by its small sample size. Another limitation of this study is its observational nature. We were not able to explore cause-effect relationship of prognostic value of high APRC levels. In addition, there were some differences with regard to aldosterone blockers and diuretics use. Patients with higher levels of APRC were more often using this kind of treatment. Although we corrected for this difference, we might not be able to show their true influence in multivariate analysis.

Conclusions

In conclusion, increased APRC is a strong predictor of outcome in patients with HF using RAAS-blocking agents. Heart failure patients with decreased kidney function and a high APRC are at the highest risk for adverse clinical outcome. Our data suggest that renin regulation and possibly renin inhibition may be of particular importance in this group of patients. Further studies are needed to answer whether additional therapy aimed at sustained RAAS activation could be beneficial in patients with HF and impaired renal function.

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Chapter 8

Summary and future perspectives

Summary

Heart failure is a clinical syndrome with high morbidity and mortality rates [1]. The effects of treatment and thus clinical outcome in heart failure patients are very often compromised by the existing co-morbidities, among which one of the most common is renal failure. Its presence significantly influences the treatment and prognosis in heart failure patients [2]. Therefore, this co-existence of heart and kidney dysfunction, often referred to as "cardiorenal syndrome", has received much attention in the last years [3,4]. Multiple mechanisms have been proposed as the underlying mechanisms of this heart-kidney interaction, among which hemodynamic changes, neurohormonal activation, inflammation and endothelial dysfunction seem to play a central role. However, the exact pathophysiology of the cardiorenal syndrome remains unclear. This thesis aimed to improve the understanding of this important clinical condition by introduction of a new animal model and imaging techniques for experimental studies and further investigation of the effects of common treatment strategies in the clinical setting.

The cardiorenal interaction has been and is currently being investigated in multiple clinical trials involving different treatment strategies [5,6,7]. However, because of the observational character of most of the studies, an in-depth investigation of the pathophysiology of the heart-kidney relationship is not fully possible. Therefore, a better understanding of this clinical condition requires well-designed animal studies performed in a dedicated animal model.

In PART I we explored the possibilities of animal studies on the cardiorenal interaction. In chapter 2 we have reviewed different animal models that have been so far used in studies on cardiorenal interaction. These animal models are based on the use of rodents, since this approach has the advantages of relatively low costs, availability, and housing conditions. Although, they all represent, to some extent, clinical characteristics of the cardiorenal syndrome, still the translation towards the clinical situation is limited. Therefore, a new and better model is needed. Such a model should meet multiple requirements as mentioned in chapter 2, including progressive deterioration of both organs, hemodynamic changes affecting both heart and kidney and activation of various compensating neurohormonal systems, and finally confirmation by histological changes as observed in patients with the cardiorenal syndrome. The recent developments in molecular biology techniques have increased the number of possibilities in creating new models for various diseases. This approach may also lead to the development of a new model for the cardiorenal syndrome. However, it has to be addressed that both heart and kidney failure are heterogeneous disorders resulting from multiple clinical conditions involving various pathophysiological pathways. Therefore, we may as well not be able to develop one model entirely mimicking the clinical characteristics of the cardiorenal syndrome. The alternative may be the use of different models, depending on the postulated research question. A more or less integrated analysis of the results obtained from multiple studies making use of various models may help us to understand the complexity of the heart-kidney interactions.

In our experimental studies on the cardiorenal interaction, presented in chapter 3 and 4, we used a novel animal model for the cardiorenal interaction. We have chosen a Munich-Wistar-Fromter (MWF) rat, a strain characterized by spontaneous and gradual development of renal dysfunction, resulting in increased proteinuria and glomerulosclerosis [8,9]. The gradual deterioration of the kidney function mimics the time-course of renal failure observed in humans. In chapter 3 we have investigated the cardiac sensitivity to acute ischemia/reperfusion damage in MWF rats in comparison to healthy Wistar rats and hypertensive SHR. It has been suggested in literature that cardiac ischemic tolerance in the presence of renal dysfunction is reduced [10], which may explain worse outcome of these patients. It is believed that next to factors associated with the kidney and circulation, also cardiac factors contribute to this reduced cardiac ischemic tolerance [11,12]. We showed increased sensitivity to ischemia in MWF rats, which could not be attributed to hypertension or adverse cardiac remodelling. Our findings are in accordance with the recent findings by Dikow and colleagues in an alternative model [13], and suggest alternative mechanisms to play a role in increased heart ischemic sensitivity in renal dysfunction. These mechanisms could include endothelial dysfunction, altered neurohumoral responsiveness and/or anemia and should be further studied in various animal models.

In chapter 4 we have studied the time-course of changes in both cardiac- and renal function in our model after introducing a myocardial infarction. We showed that experimental myocardial infarction did not exaggerate renal function, however, the deterioration of cardiac function was accelerated by the presence of renal dysfunction. Renal dysfunction in our model was confirmed by increased proteinuria and histological changes. Interestingly, creatinine clearance was not different from values observed in other groups. This seems to support the hypothesis that proteinuria and reduced glomerular filtration rate represent different types of renal dysfunction [14]. Again, this underscores the heterogeneous character of renal failure, which should be considered when analyzing the data from experimental studies. Interestingly, with regard to cardiac function, we have observed not only systolic, but also prominent diastolic dysfunction. This is in agreement with clinical observations in patients with renal dysfunction [15]. Moreover, recently it has been suggested that diastolic dysfunction develops into congestive diastolic heart failure because of underlying renal insufficiency [16]. We suggest that this model could serve as a tool for further investigation of this interesting and clinically important aspect of the cardiorenal interaction. Our study showed that in this model endothelial dysfunction is an important mediator in the cardiorenal interaction. There is growing evidence of the role of endothelium in regulation of cardiac systolic and diastolic function [17,18], as well as renal perfusion in heart failure patients [19]. Endothelial function also plays a crucial role in the actions of erythropoietin [20,21]. Since the correction of anemia improves both cardiac and renal function in heart failure [22], endothelial dysfunction might be an important contributor in the heart/kidney interaction. Future research is needed in order to evaluate the exact role of endothelial dysfunction in the pathophysiology of the cardiorenal syndrome, its relevance in

the clinical setting and potentially beneficial treatment strategies.

The animal studies on the cardiorenal interaction require not only a relevant animal model, but also tools to investigate various aspects of the heart-kidney connection. Especially the proper quantification of hemodynamic changes and organ perfusion remain a challenge. The currently used techniques are not suitable for repetitive measurements due to their invasive character. In PART II we have studied positron emission tomography (PET) with the use of ^{13}N-NH_3 as a method of evaluation of the cardiorenal axis, which can be used in experimental studies. Introduction of PET allowed quantitative and non-invasive measurements of multiple organs function and perfusion. PET scanning with ^{13}N-NH_3 has been used in the evaluation of cardiac and renal perfusion in humans [23,24]. Promising results have been obtained also for simultaneous measurements of myocardial perfusion, left ventricle volumes (LVV) and ejection fraction (LVEF) with the use of ^{13}N-NH_3 PET [25]. In chapter 5 we showed that this technique allows measurement of both LVV and LVEF simultaneously in healthy rats and rats after myocardial infarction and results obtained with this approach are comparable with the measurements with the golden standard, ^{18}F-FDG micro-PET. Moreover, we showed that it might be used for repetitive measurements, which allows monitoring of the changes in measured parameters over time. This approach thus offers a tool for monitoring the progression of disease and the efficacy of pharmaceutical and non-pharmaceutical interventions.

The hemodynamic changes in heart failure resulting from decreased cardiac output lead to reduction of organ perfusion, including the kidneys [26]. It has been hypothesized that restoration of cardiac function would lead to an improvement in renal perfusion, and thus renal function [27]. However, the tool for repetitive measurements of regional renal perfusion in small animals is still missing, and therefore the research possibilities regarding this aspect of cardiorenal interaction are limited. In chapter 6 we described a pilot study with healthy rats and rats with myocardial infarction, in which we studied the use of ^{13}N-NH_3 micro-PET for simultaneous assessment of both cardiac and renal regional perfusion. We showed that experimental cardiac dysfunction leads to reduced cardiac perfusion and we found a trend towards a reduction in renal perfusion. The latter was more pronounced in the cortical regions, supporting findings from literature [28,29]. Interestingly, these changes correlated with histological changes. As expected, measured cardiac perfusion was strongly correlated with infarct size and LVEF. Our results suggest that micro-PET with the use of ^{13}N-NH_3 might serve as an useful non-invasive tool for simultaneous measurement of cardiac and regional renal perfusion in small animal models. This technique might be used in studies on renal perfusion in the presence of cardiac dysfunction. In addition, it may provide more insight into mechanisms activated by different treatment strategies. Implementation of this technique in experimental studies could also help in comparing results from the animal studies on cardiorenal interaction with the clinical setting, this would underscore the similarities and differences between these studies and therefore could lead to easier translation and better understanding of the cardiorenal syndrome.

One of the most important cardiorenal connectors is the renin-angiotensin-aldosterone system

(RAAS) [3,4]. We further explored the role of the RAAS in heart failure patients in PART III. Since the pharmacological blockade of the RAAS resulted in improved prognosis [30,31], it has become one of the cornerstones of the therapy in heart failure patients. However, long-term RAAS blockade disables regulatory mechanisms of the kidney and can lead to further decrease in renal blood flow and GFR. Moreover, RAAS-blocking agents increase the plasma levels of prorenin and renin. In chapter 7 we studied whether this increase might be of prognostic value. We have studied a relatively small group of heart failure patients being treated with ACE inhibitors. We showed that renin is indeed a strong prognostic factor in this patient population. This is in agreement with previous data from literature [32]. Interestingly, the prognostic value of renin was especially pronounced in the group of patients with reduced kidney function. Even after adjustment for various kidney function parameters, renin remained a significant predictor of outcome. Interestingly, prorenin did not show prognostic value in our study. The association between increased renin levels and worse outcome might be a reflection of ACE escape mechanisms, reported before in literature [33]. Another possibility is the action exerted by renin independently of angiotensin formation. This hypothesis is supported by the recent findings on prorenin/renin receptor [34,35]. Our results suggest that beneficial effects could be achieved by blocking the RAAS at the level of renin, especially in patients with decreased renal function. This can be achieved by the use of direct renin inhibitors. They have already been shown to have favorable effects on neurohormonal status in patients with HF [36] and are currently studied in multiple trials, such as ATMOSPHERE, ALTITUDE and ARIANA-CHF [6,7]. Whether these agents could serve as an additional treatment in heart failure patients needs further clarification and we need to await the results from ongoing trials to be able to answer this question.

Future perspectives

In this thesis we have addressed multiple aspects of experimental and clinical studies on the cardiorenal interaction, which should be further investigated.

We used an animal model for studies on combined heart- and kidney failure. Our studies suggest an important role of endothelial dysfunction in the cardiorenal interaction. Although it has already been known that endothelial dysfunction is present in the heart failure population and is associated with renal dysfunction, our results suggest that it may play an important mediating role in development of heart/renal failure in the presence of the disorder of one of the organs. Future studies on endothelial dysfunction and its interaction with other cardiorenal connectors as well as on possible treatment strategies are therefore required.

Our studies on imaging of the cardiorenal axis with ^{13}N-NH_3 micro-PET show that this method offers an attractive alternative to invasive measurements used so far. We demonstrated that with the use of this technique, quantitative, repetitive measurements of left ventricle function

and volumes can be obtained. Our pilot study results also suggest that regional kidney perfusion as well as myocardial perfusion may be obtained non-invasively with this technique. The latter has to be confirmed in larger studies. This could lead to a significant improvement of our understanding of the different cardiorenal interactions in heart failure patients.

The important role of the RAAS in the cardiorenal interaction has been addressed multiple times. RAAS blockade has become one of the cornerstones of heart failure treatment and resulted in reduced mortality. However, it also leads to and increase in renin levels, which we showed to correlate with worse outcome, especially in the group of patients with impaired kidney function. This suggest that more beneficial effects could be obtained in this group of patients with the use of direct renin blockers. Currently ongoing and future clinical trials with these agents should give the answer whether they can be a significant improvement in the therapy.

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Nederlandse samenvatting

Samenvatting

Hartfalen is een klinisch syndroom dat geassocieerd is met een hoge morbiditeit en mortaliteit. De effecten van de behandeling en dus de klinische uitkomst bij patiënten met hartfalen zijn vaak beperkt door de aanwezigheid van andere aandoeningen, waarvan nierfalen één van de meest voorkomende is. Nierfalen heeft grote invloed op de behandeling en de prognose bij hartfalen patiënten. Daarom heeft deze co-existentie van hart- en nierdysfunctie, ook wel het "cardiorenaal syndroom" genoemd, veel aandacht gekregen in de afgelopen jaren. Meerdere onderliggende mechanismen lijken een rol te spelen in dit gezamenlijk falen van hart en nier. Hemodynamische veranderingen, neurohormonale activatie, ontsteking en endotheeldysfunctie lijken de meest prominente rol te spelen. Echter, de onderliggende pathofysiologie van het cardiorenaal syndroom is onduidelijk. Dit proefschrift is erop gericht om de kennis van en begripsvorming over de cardiorenale interactie in het kader van hartfalen te vergroten door de introductie van een nieuw diermodel en beeldvormende technieken voor experimentele studies en aanvullend onderzoek in patiënten met hartfalen naar de effecten van de vaak gebruikte behandelstrategieën.

De cardiorenale interactie werd en wordt momenteel onderzocht in meerdere klinische studies waarbij onder andere gekeken wordt naar verschillende therapeutische strategieën. Echter, als gevolg van het observationele karakter van de meeste van deze studies is mechanistisch onderzoek naar de pathofysiologie van de hart-nier-relatie niet goed mogelijk. Een beter begrip van het cardiorenaal syndroom zou verkregen moeten worden in goed opgezette, gecontroleerde experimenten in het juiste diermodel.

In DEEL I hebben we de mogelijkheden voor dierexperimenten op het gebied van de cardiorenale interactie onderzocht. In hoofdstuk 2 zijn de verschillende diermodellen die tot nu toe gebruikt zijn gebruikt in studies, met als onderwerp de cardiorenale interactie, besproken in hun pathofysiologische context. Als diermodel werd in het merendeel van de gevallen gebruik gemaakt van een knaagdiermodel wat het voordeel heeft van relatief lage kosten, ruime beschikbaarheid en controleerbare huisvestingcondities. Hoewel deze diermodellen enigszins de klinische kenmerken van het cardiorenaal syndroom laat zien blijft de vertaling van de bevindingen naar de klinische praktijk moeizaam en beperkt. Daarom is er dringend behoefte aan nieuwe en betere modellen. Een dergelijk model moet kenmerken bevatten zoals beschreven in hoofdstuk 2. Hierbij moet men denken aan een progressieve verslechtering van de functie van beide organen, hemodynamische veranderingen die zowel het hart als de nieren beïnvloeden, activatie van de verschillende neurohormonale systemen en histologische veranderingen zoals waargenomen bij patiënten met het cardiorenaal syndroom. Door de recente stormachtige ontwikkelingen van nieuwe moleculair biologische technieken zijn de mogelijkheden van translationeel onderzoek enorm vergroot. Dit kan ook leiden tot de ontwikkeling van een nieuw model voor het cardiorenaal syndroom. Echter, hart- als nierfalen zijn heterogene syndromen en zijn het gevolg van verschillende klinische aandoeningen. Daarom

is het bijzonder moeilijk om een model te ontwikkelen die alle klinische kenmerken van het cardiorenaal syndroom in zich heeft. Een alternatief is om hiervoor verschillende modellen in te zetten, die gerelateerd zijn aan een specifieke onderzoeksvraag. Een geïntegreerde analyse van de resultaten van verschillende modellen zou ons dan mogelijk verder kunnen helpen om de complexiteit van de interactie tussen hart en nier beter te leren begrijpen.

In de experimentele studies in hoofdstuk 3 en 4, hebben we gebruik gemaakt van een nieuw diermodel voor de cardiorenale interactie. Er is hier gekozen voor de Munich-Wistar-Fromter (MWF) rat, een stam die wordt gekenmerkt door de spontane ontwikkeling van renale dysfunctie, wat leidt tot een verhoogde proteïnurie en glomerulosclerose. De geleidelijke verslechtering van de nierfunctie lijkt op het beloop van nierfalen zoals dit ook wordt gezien bij patiënten met nierfalen. In hoofdstuk 3 hebben we de cardiale gevoeligheid voor acute ischemie / reperfusie schade in MWF ratten onderzocht en vergeleken met gezonde en spontane hypertensieve ratten. In de literatuur is beschreven dat de ischemische tolerantie verminderd is in aanwezigheid van nierdysfunctie wat de slechtere prognose van deze patiënten zou kunnen verklaren. Er wordt aangenomen dat naast de factoren, die geassocieerd zijn met de nieren en circulatie, ook cardiale factoren bijdragen aan een verminderde cardiale ischemische tolerantie. We toonden een verhoogde gevoeligheid voor ischemie aan in MWF ratten die niet verklaart kon worden door de aanwezigheid van hypertensie of cardiale remodellering. Onze bevindingen zijn in overeenstemming met recente bevindingen van Dikow in een ander model en suggereren de aanwezigheid van alternatieve paden. Deze paden kunnen endotheeldysfunctie, veranderde neurohumorale gevoeligheid en/of anemie zijn. Deze mechanismen moeten verder onderzocht worden in verschillende diermodellen.

In hoofdstuk 4 hebben we de veranderingen in hart-en nierfunctie na een hartinfarct onderzocht. We zagen dat een experimenteel hartinfarct de nierfunctie niet beïnvloedt, maar dat de verslechtering van de hartfunctie wel werd versneld door de aanwezigheid van een verminderde nierfunctie. De nierdysfunctie in dit model werd bevestigd door de aanwezigheid van proteïnurie en histologische veranderingen. Interessant in ons experiment was de bevinding dat creatinineklaring niet verschillend was tussen de verschillende groepen. Dit lijkt onze hypothese te ondersteunen dat proteïnurie en een verminderde glomerulaire filtratiesnelheid verschillende entiteiten van nierdysfunctie zijn. Verder, bevestigt dit nogmaals het heterogene karakter van nierfalen. Dit heterogene karakter moet in ogenschouw worden genomen bij de interpretatie van gegevens verkregen uit experimenteel onderzoek. Een opvallende bevinding was verder dat de cardiale dysfunctie niet alleen de systolische, maar ook de diastolische dysfunctie betrof. Dit is in overeenstemming met klinische observaties bij patiënten met een verminderde nierfunctie. Recent is gesuggereerd dat diastolische dysfunctie kan leiden tot diastolisch hartfalen door onderliggende nierfalen. Ons dierexperimenteel model zou een instrument kunnen zijn in verder onderzoek naar dit interessante en klinisch belangrijk aspect in de cardiorenale interactie. In ons model was de endotheeldysfunctie één van de belangrijkste determinanten van de cardiorenale interactie. Er zijn steeds meer

aanwijzingen dat het endotheel daarnaast een rol speelt in de regulatie van, speelt ook een rol in de regulatie de systolische en diastolische hartfunctie zowel in de regulatie van renale perfusie bij patiënten met hartfalen. Endotheelfunctie speelt verder ook een cruciale rol in de werking van het hormoon erytropoëtine dat een specifieke stimulerende werking heeft op de vorming van rode bloedcellen. Omdat een verhoging van het aantal rode bloedcellen bij patiënten met te weinig rode bloedcellen zowel de hart- als nierfunctie bij hartfalen verbetert, is het te verwachten dat het endotheel een belangrijke rol speelt in de driehoek hart, nier en anemie, ook wel het cardiorenale anemie syndroom genoemd. Verder onderzoek is nodig om de precieze rol van endotheel dysfunctie in de pathofysiologie van het cardiorenale anemie syndroom, de relevantie ervan in de klinische setting en de potentiële therapeutische strategieën te onderzoeken.

Dierexperimenten naar de cardiorenale interactie vereisen niet alleen een adequaat model, maar ook specifieke meetinstrumenten om de verschillende aspecten van de interactie tussen hart en nier te kunnen onderzoeken. Vooral de kwantificatie van hemodynamische veranderingen en perfusie van de beide organen zijn een uitdaging. Vanwege hun invasieve karakter zijn de op dit moment gebruikte technieken niet geschikt voor het uitvoeren van herhaalde metingen.

In DEEL II bestudeerden we in een dierexperimenteel model de mogelijkheden van positronemissietomografie (PET) met ^{13}N-NH_3 voor de afbeelding en evaluatie van de cardiorenale as.. Met PET is het mogelijk om van meerdere organen tegelijkertijd kwantitatieve en niet-invasief orgaanfunctie en orgaanperfusie te meten . PET-scanning met ^{13}N-NH_3 is bij mensen al succesvol gebruikt voor de evaluatie van cardiale en renale perfusie. Goede resultaten met ^{13}N-NH_3 PET zijn ook verkregen tijdens gelijktijdige metingen van myocard perfusie, linker ventrikel volumes (LVV) en ejectiefractie (LVEF). In hoofdstuk 5 hebben we aangetoond dat deze techniek gebruikt kan worden voor metingen van zowel LVV als LVEF bij gezonde ratten en ratten met myocardinfarct. De resultaten zijn vergelijkbaar met de metingen met de gouden standaard, ^{18}F-FDG micro-PET. We hebben ook aangetoond dat deze techniek gebruikt kan worden voor herhaalde metingen. Dit maakt de monitoring van veranderingen in de gemeten parameters in de tijd mogelijk. Deze aanpak biedt dus een instrument voor onderzoek naar de progressie van de ziekte en de werking van farmaceutische en niet-farmaceutische interventies. De hemodynamische veranderingen bij hartfalen als gevolg van een verminderde cardiale output leiden tot een vermindering van de perfusie van de organen, inclusief de nieren. Het is verondersteld dat het herstel van de hartfunctie zou leiden tot een verbetering van de renale perfusie, en dus de nierfunctie. Een meetinstrument waarmee we regionale renale perfusieveranderingen van organen in de tijd kunnen meten in kleine dieren ontbreekt op dit moment. Om die reden zijn de mogelijkheden voor dit type onderzoek met betrekking tot de cardiorenale interactie beperkt.

In hoofdstuk 6 beschrijven we een pilot-studie met gezonde ratten en ratten met een hartinfarct, waarin we de waarde van ^{13}N-NH_3 micro-PET onderzocht hebben voor een gelijktijdige

beoordeling van zowel hart- als renale regionale perfusie. We lieten zien dat experimentele cardiale dysfunctie leidt tot een verminderde cardiale perfusie en we zagen een trend in een vermindering van de renale perfusie. De laatste bevinding was meer uitgesproken in de corticale gebieden van de nier, wat ondersteund wordt door bevindingen uit de literatuur. Een belangrijke bevinding was dat deze veranderingen correleerden met histologische veranderingen. Zoals verwacht, was de gemeten cardiale perfusie sterk gecorreleerd met de grootte van het infarct en de LVEF. Onze resultaten suggereren dat micro-PET met het gebruik van ^{13}N-NH_3 zou kunnen dienen als een bruikbare niet-invasieve techniek voor gelijktijdige metingen van cardiale and regionale renale perfusie in kleine diermodellen. Deze techniek kan inzicht geven hoe de perfusie van de nier veranderd bij het optreden hartfalen. Daarnaast kan deze techniek meer inzicht geven in de werkingsmechanismen en impact van verschillende medicamenteuen niet-medicamenteuze behandelingsstrategieën. Een vergelijking met observaties uit de klinische praktijk ligt voor de hand. Translationele overeenkomsten en verschillen zouden kunnen leiden tot een beter begrip van het cardiorenaal syndroom.

Één van de belangrijkste cardiorenale connectors is het renine-angiotensine-aldosteron systeem (RAAS). We hebben de rol van het RAAS bij patiënten met hartfalen verder onderzocht in DEEL III. Omdat de farmacologische blokkade van het RAAS leidde tot een verbeterde prognose in patiënten met hartfalen werd blokkade van het RAAS één van de hoekstenen van de therapie bij deze patiënten. Echter, op de lange termijn ontregelt chronische RAAS blokkade de regulatie mechanismen van de nieren wat kan leiden tot verdere daling van de nierperfusie en GFR. Daarnaast verhogen RAAS-remmers de plasmaspiegels van prorenin en renine.

In hoofdstuk 7 hebben we onderzocht of deze stijging een prognostische betekenis zou kunnen hebben. In een relatief kleine groep van patiënten met hartfalen die behandeld worden met ACE-remmers toonden we aan dat renine een sterke prognostische factor op het optreden van aan het hart gerelateerde ongewenste gebeurtenissen. Dit is in overeenstemming met eerdere data uit de literatuur. Interessant is dat de prognostische waarde van renine vooral zichtbaar was in de groep patiënten met een verminderde nierfunctie. Zelfs na correctie voor diverse aan nierfunctie gerelateerde parameters, bleef renine een significante voorspeller van prognose. Opvallend is dat prorenine geen prognostische waarde had in onze studie. Het verband tussen verhoogde renine spiegels en een slechtere klinische uitkomst zou een weerspiegeling kunnen zijn van ACE escape mechanismen, een fenomeen welke al eerder beschreven is in de literatuur. Een andere mogelijkheid is dat de werking van renine onafhankelijk is van angiotensinevorming. Deze hypothese wordt ondersteund door recente bevindingen over de prorenine / renine-receptor. Onze resultaten suggereren dat gunstige effecten vooral bij patiënten met een verminderde nierfunctie zouden kunnen worden bereikt door het blokkeren van het RAAS op het niveau van renine. Dit zou bijvoorbeeld kunnen worden bewerkstelligd door het gebruik van directe renineremmers. Deze middelen hebben al eerder gunstige effecten laten zien op de neurohormonale status bij patiënten met HF. Directe renine blokkers worden momenteel onderzocht in verschillende studies, zoals ATMOSPHERE, ALTITUDE en ARIANA-CHF. Of

deze middelen hun toepassing hebben bij patiënten met gecombineerd hart en nierfalen is nog onbekend.

Toekomstperspectieven

In dit proefschrift is translationeel de cardiorenale interactie onderzocht.
In het experimentele deel beschrijven we een nieuw diermodel van gecombineerd hart- en nierfalen. Onze studies beschrijven een belangrijke rol voor endotheeldysfunctie in de cardiorenale interactie en er kan worden geconcludeerd dat endotheeldysfunctie waarschijnlijk een belangrijke rol speelt in de ontwikkeling van gecombineerd hart- en nierfalen. Toekomstige studies over endotheeldysfunctie, de interactie met andere cardiorenale connectors en over mogelijke therapeutische strategieën zijn dan ook noodzakelijk.
De studies over beeldvorming van de cardiorenale as met ^{13}N-NH_3 micro-PET laten zien dat deze methode een aantrekkelijk alternatief voor invasieve metingen zou kunnen zijn. We hebben aangetoond dat met deze techniek kwantitatieve herhaalde metingen van de linker ventrikelfunctie en volume mogelijk zijn. Onze pilot-studie suggereerde dat met behulp van deze techniek regionale nierperfusie en myocardperfusie niet-invasief gemeten kunnen worden. De klinische relevantie dient te worden uitgezocht in grotere studies. Dit kan leiden tot een substantiële verbetering van ons inzicht in de verschillende cardiorenale interacties bij patiënten met hartfalen.
De rol van het RAAS in de cardiorenale interactie is al meerdere keren onderzocht. RAAS blokkade is één van de hoekstenen van de hartfalen behandeling, wat onder andere heeft geresulteerd in een verminderde mortaliteit. Echter; blokkade van het RAAS leidt ook tot een toename van de renine spiegels. Wij hebben aangetoond dat deze hoge renine spiegels correleren met slechtere prognose, vooral in de groep patiënten met een verstoorde nierfunctie. Dit suggereert dat in het bijzonder deze groep patiënten baat zouden kunnen hebben bij het gebruik van directe renine blokkers. Studies welke op dit moment worden uitgevoerd en toekomstige klinische studies met deze geneesmiddelen zullen antwoorden geven of deze middelen daadwerkelijk een verbetering van therapie zou kunnen zijn.

Streszczenie

Streszczenie

Niewydolność serca to choroba charakteryzująca się wysoką zachorowalnością i śmiertelnością. Efektywność leczenia pacjentów z niewydolnością serca jest często ograniczona poprzez współwystępujące choroby, spośród których jedną z najczęściej występujących jest niewydolność nerek. Ponieważ współwystępowanie niewydolności serca i nerek, nazywane "zespołem secowo-nerkowym (kardionefrologicznym)" wiąże się z gorszym rokowaniem, stało się ono przedmiotem wielu badań naukowych w ostatnich latach. Uważa się, iż u podstaw tego zespołu leżą liczne mechanizmy regulacyjne, spośród których centralną rolę odgrywają zmiany hemodynamiczne, aktywacja układów nerwowo-hormonalnych, zapalenie i niewydolność śródbłonka. Jednakże, dokładne powiązania patofizjologiczne sa wciąż nieznane. Celem niniejszej pracy było pogłębienie wiedzy na temat tego istotnego klinicznie zespołu chorobowego poprzez badania z zastosowaniem nowego modelu zwierzęcego, nowych technik obrazowania oraz analizę wpływu stosowania popularnych leków na rokowanie pacjentów z niewydolnością serca.

Zespół sercowo-nerkowy jest analizowany w wielu prowadzonych obecnie badaniach klinicznych z zastosowaniem różnych modeli terapeutycznych. Jednak obserwacyjny charakter tych badań nie pozwala na dokładną analizę patofizjologicznych powiązań pomiędzy sercem i nerkami. Ten cel można osiągnąć poprzez badania eksperymentalne z zastosowaniem zwierząt laboratoryjnych. Część I dotyczy takich właśnie badań. W rozdziale 2 przeanalizowaliśmy i podsumowaliśmy różne modele zwierzęce, zastosowane dotychczas w badaniach kardionefrologicznych. Pomimo iż wszystkie te modele posiadają w pewnym stopniu cechy zespołu chorobowego obserwowanego u pacjentów, wyniki uzyskane w tych badaniach nie zawsze mogą być zastosowane w praktyce klinicznej. Z tego powodu potrzebny jest nowy model zwierzęcy do badań eksperymentalnych. W rozdziale 2 przedstawiliśmy cechy, jakimi powinnien się on charakteryzować, takie jak stopniowy rozwój niewydolności obu narządów, zmiany hemodynamiczne i aktywacja różnych neurohormonalnych mechanizmów regulacyjnych, jak również zmiany histologiczne obserwowane w populacji pacjentów. Jednym ze sposobów uzyskania takiego modelu może być zastosowanie ostatnich osiągnięć biologii molekularnej Należy jednak pamiętać, że zarówno niewydolność serca jak i nerek to zespoły chorobowe, które są wynikiem różnych chorób pierwotnych i aktywacji licznych mechanizmów patofizjologicznych. Z tego powodu stworzenie jednego modelu zwierzęcego, który w pełni byłby odzwierciedleniem obserwacji klinicznych może okazać się niemożliwe. Alternatywą może być zastosowanie różnych modeli, w zależności od badanego zagadnienia. Jednoczesna analiza wyników takich badań może pomóc w lepszym zrozumieniu powiązań między sercem i nerkami.

W rozdziale 3 i 4 prezentujemy wyniki badań eksperymentalnych, w których zastosowaliśmy nowy model zwierzęcy oparty na szczurach szczepu Wistam Munich Fromter (MWF). Zwierzęta te charakteryzują się stopniowym rozwojem niewydolności nerek prowadzącym do

białkomoczu i szkliwienia kłębuszków nerkowych. W rozdziale 3 badaliśmy wrażliwość mięśnia sercowego na zmiany niedokrwienne w naszym modelu w porównaniu do innych modeli – zdrowych szczurów Wistar i szczurów z nadciśnieniem tętniczym (SHR). Dotychczasowe badania sugerują, iż niewydolność nerek prowadzi do zwiększonej wrażliwości mięśnia sercowego na zmiany niedokrwienne, jakkolwiek czynniki związane z samym mięśniem sercowym również odgrywają znaczącą role. W naszych badaniach wykazaliśmy, iż zmiany histologiczne w mięśniu sercowym, czy też nadciśnienie tętnicze nie wyjaśniają w wystarczającym stopniu zmniejszonej tolerancji miokardium na zmiany niedokrwienne. Nasze wyniki potwierdzają wcześniejsze doniesienia i sugerują, iż inne niż dotychczas uważano czynniki odgrywają rolę w powyższym procesie.

W rozdziale 4 opisane są badania nad zmianami w wydolności serca i nerek następujące po długotrwałym niedokrwieniu mięśnia sercowego, czyli zawale serca. W badaniach tych wykazaliśmy, iż w naszym modelu niewydolność serca po zawale serca nie miała znaczącego wpływu na funkcję nerek, jednak współistnienie niewydolności nerek i serca przyspieszyło rozwój tej drugiej. Dysfunkcja nerek w naszym badaniu charakteryzowała się obecnością białkomoczu i zmian histologicznych, jednakże klirens kreatyniny nie był obniżony, co potwierdza różnorodność obrazów klinicznych obserwowanych w niewydolności nerek. Jesli chodzi o niewydolność serca, to w naszych badaniach wykazaliśmy obecność zarówno niewydolnośći skurczowej jak i rozkurczowej serca, co odzwierciedla obserwacje kliniczne. Co ciekawe, niektórzy autorzy sugerują, iż współistnenie niewydolności nerek jest niezbędnym warunkiem do rozwoju rozkurczowej niewydolności serca. Zastosowany przez nas model może być przydatny w dalszych badaniach nad tym aspektem zespołu kardionefrologicznego. Nasze wyniki sugerują, iż ważnym elementem w patofizjologii powiązań pomiędzy sercem i nerkami jest (dys)funkcja śródbłonka. Ostatnie badania wskazują na ważną rolę śródbłonka w regulacji funkcji serca, zarówno skurczowej jak i rozkurczowej, a także perfuzji nerek u pacjentów z niewydolnością serca. Zachowana funkcja śródbłonka jest istotna także dla prawidłowego działania erytropoetyny, co dodatkowo podkreśla jej istotną rolę w zespole kardionefrologicznym, ponieważ leczenie niedokrwistości u pacjentów z niewydolnością serca poprawia funkcję serca i nerek. Dlatego też dalsze badania powinny skoncentrować się na ustaleniu dokładnej roli (dys)funkcji śródbłonka w relacji pomiędzy sercem a nerkami, co może doprowadzić do wprowadzenia nowych strategii terapeutycznych.

Badania eksperymentalne nad zespołem sercowo-nerkowym wymagają nie tylko zastosowania odpowiedniego modelu zwierzęcego, ale również technik pozwalających na pomiary ilościowe i jakościowe zmian patofizjologicznych zachodzących w trakcie tych badań. Obecnie największym wyzwaniem pozostają dokładne pomiary zmian hemodynamicznych i zmian w ukrwieniu różnych narządów. Ze względu na ich inwazyjny charakter, techniki stosowane obecnie nie mogą być używane do wielokrotnych pomiarów. W części II zanalizowaliśmy możliwości zastosowania pozytronowej tomografii emisyjnej (positron emission tomography, PET) z zastosowaniem ^{13}N-NH_3 w badaniach eksperymentalnych. Wprowadzenie PET

pozwoliło na nieinwazyjne pomiary zarówno funkcji jak i ukrwienia wielu narządów. PET z użyciem ^{13}N-NH_3 jako znacznika jest używany do pomiarów perfuzji miokardium i nerek u ludzi. Obiecujące wyniki dały także badania nad jednoczesnym pomiarem perfuzji miokardium, geometrii i funkcji serca. W rozdziale 5 pokazaliśmy, iż zastosowanie tej techniki pozwala na jednoczesne pomiary geometrii i funkcji serca szczurów, zarówno zdrowych jak i szczurów z niewydolnością serca. Wyniki uzyskane przy pomocy tej metody były porównywalne z tymi uzyskanymi za pomocą metody uznanej za złoty standard, czyli skanów z użyciem ^{18}F-FDG jako znacznika. Dodatkowo, pokazaliśmy iż metoda ta pozwala na wielokrotne pomiary u jednego osobnika, co ma kluczowe znaczenie dla pokazania ewentualnych zmian badanych parametrów w czasie. Dzięki temu możliwe staje się monitorowanie zmian chorobowych jak i efektów zastosowania różnych terapii w badaniach eksperymentalnych.

Zmiany hemodynamiczne obserwowane w niewydolności serca są efektem zmniejszonego rzutu serca i prowadzą do zmniejszonej perfuzji narządów obwodowych, w tym nerek. Uważa się, iż poprawa funkcji serca może doprowadzić do zwiększenia ukrwienia nerek, a w konsekwencji do poprawy ich funkcji. Jednakże obecnie brakuje narzędzi eksperymentalnych pozwalających na wielokrotne pomiary ukrwienia nerek, przez co badania nad tym zjawiskiem są ograniczone. W rozdziale 6 opisujemy wstępne badania, w których analizowaliśmy zastosowanie PET z ^{13}N-NH_3 jako znacznikiem do jednoczesnych pomiarów ukrwienia mięśnia sercowego i nerek. W badaniach tych zaobserowaliśmy zmniejszone ukrwienie mięśnia sercowego i trend w kierunku zmniejszonej perfuzji nerek u zwierząt z niewydolnością serca. Zmniejszenie ukrwienia nerek było głównie obserwowalne w regionach korowych, zgodnie z doniesieniami z innych badań. Co ciekawe, zmiany te były skorelowane z zaobserowanymi zmianami strukturalnymi. Zgodnie z oczekiwaniami, ukrwienie mięśnia sercowego było silnie powiązane z wielkością obszaru objętego niedokrwieniem jak i z funkcją skurczową serca. Nasze wyniki sugerują, iż technika ta może być przydatnym narzędziem do oceny ukrwienia obu narządów. Jej zastosowanie w badaniach eksperymentalnych może pomóc w zrozumieniu zmian zachodzących w niewydolności serca, pozwoli porównać wyniki uzyskiwane w badaniach nad zwierzętami z wynikami z badań klinicznych, a co za tym idzie pomoże w lepszym zrozumieniu patofizjologii zespołu kardionefrologicznego.

Jednym z najważniejszych elementów w patofizjologii wzajemnych połączeń pomiędzy sercem a nerkami jest układ renina-angiotensyna-aldosteron (renin-angiotensin-aldosterone system; RAAS). W części III prezentujemy wyniki analizy roli RAAS w opisywanym zespole. Farmakologiczna blokada RAAS doprowadziła do poprawy rokowania u pacjentów, przez co stała się ona jedną z podstawowych strategii terapeutycznych w niewydolności serca. Jednakże długotrwałe blokowanie RAAS prowadzi do dysfunkcji mechanizmów regulacyjnych nerki, a co za tym idzie zmniejszenia ich funkcji. Ponadto, ze względu na pętle sprzężenia zwrotnego, prowadzi ona do wzostu poziomów proreniny i reniny we krwi. W rozdziale 7 zanalizowaliśmy czy ten wzrost może mieć wpływ na rokowanie pacjenów. Badanie zostało przerpowadzone w relatywnie małej grupie pacjenów leczonych inhibitorami konwertazy angiotensyny (ACE). Z naszej analizy wynika, iż faktycznie wysokie poziomy reniny są czynnikiem decydującym o

rokowaniu, co potwierdza doniesienia z innych badań. Ponadto, zależność ta była najsilniej wyrażona w grupie pacjentów z obniżoną funkcją nerek. Nie stwierdziliśmy przy tym podobnego wpływu podwyższonych poziomów proreniny na rokowanie w tej grupie pacjentów. Związek reniny z gorszym rokowaniem pacjentów może wynikać z opisanego wcześniej "mechanizmu ucieczki", lub też z działania reniny poprzez receptor dla reniny i proreniny. Nasze wyniki sugerują, że blokada RAAS na poziomie reniny może przynieść dodatkowe korzyści, zwłaszcza w grupie pacjentów ze współistniejącą niewydolnością nerek. Można to osiągnąc poprzez stosowanie bezpośrednich inhibitorów reniny. Ich korzystny wpływ na status neurohormonalny u pacjentów z niewydolnością serca został opisany w literaturze i jest obecnie analizowany w wielu badaniach klinicznych, takich jak ATMOSPHERE, ALTITUDE czy też ARIANA-CHF. Badania te powinny wyjaśnić czy leki te mogą posłużyć jako dodatkowa terapia u pacjentów z niewydolnością serca.

Przyszłe badania

W niniejszej publikacji opisane są eksperymentalne i kliniczne badania nad różnymi aspektami zależności pomiędzy funkcją serca i nerek. Wyniki tych analiz zachęcają do dalszych badań w przyszłości.

Nasze eksperymentalne badania z zastosowaniem zwierzęcego modelu sugerują ważną rolę dysfunkcji śródbłonka w badanym przez nas zespole kardionefrologicznym. Pomimo, iż współistnienie dysfunkcji śródbłonka u pacjentów z niewydolnością serca i jej związek z funkcją nerek była już opisana wcześniej, nasze wyniki sugerują jej znacznie ważniejszą rolę w patofizjologii zespołu sercowo-nerkowego niż pierwotnie uważano. Niezbędne są dalsze badania nad tym aspektem badanej przez nas jednostki chorobowej, gdyż mogą one doprowadzić do modyfikacji strategii terapeutycznych.

Nasze badania nad pozytronową tomografią emisyjną z zastosowaniem ^{13}N-NH_3 jako znacznika pokazały, że technika ta może służyć jako alternatywa dla tych stosowanych obecnie. Dzięki jej użyciu możliwe są wielokrotne, ilościowe pomiary funkcji i geometrii serca, jak również nieinwazyjna ocena ukrwienia nerek i mięśnia sercowego. Potwierdzenie naszych wyników w większych badaniach pozwoli na rozpowszechnienie użycia tej techniki, co może doprowadzić do lepszego zrozumienia zmian patofizjologicznych zachodzących w niewydolności serca.

Znacząca rola RAAS w patofizjologii zespołu sercowo-nerkowego była udowodniona wielokrotnie, przez co blokada tego układu stała się podstawą terapii pacjentów z niewydolnością serca. Dzięki temu rokowanie tych pacjentów uległo znacznej poprawie. Jednakże jak wykazaliśmy, długotrwała blokada tego układu prowadzi do wzrostu poziomów reniny, który to wzrost ma negatywny wpływ na rokowanie. Zależność ta sugeruje, iż zastosowanie bezpośrednich inhibitorów reniny może być wyjątkowo korzystne w tej grupie pacjentów. Badania kliniczne, zarówno te prowadzone obecnie jak i przyszłe, powinny odpowiedzieć na pytanie czy zastosowanie tych leków może faktycznie przynieść dodatkowe korzyści.

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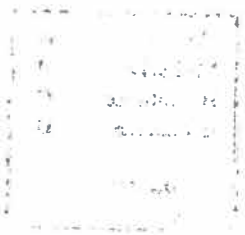

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